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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: October 15, 2009

SUBJECT: Aminocyclopyrachlor and aminocyclopyrachlor-methyl DERs

PC Codes: 288008, 288009
Decision Nos.: 400967, 400972
Petition No.: None
Risk Assessment Type: Tox DERs
TXR No: 0055188
MRID Nos: (see below)

DP Barcodes: D361080, D351256
ID No.: NA
Regulatory Action: None
Case No.: NA
CAS No.: NA
40 CFR: NA

Ver.Apr.08

FROM: Jessica Ryman, Ph.D., Toxicologist Jessica Ryman Ph.D. 10/5/2009
Risk Assessment Branch IV
Health Effects Division, 7509P

THROUGH: Susan Hummel, Senior Scientist Susan Hummel
Risk Assessment Branch IV
Health Effects Division, 7509P

TO: Mindy Ondish
Fumigants Branch
Registration Division (7505P)

I. CONCLUSIONS

The DERs comprising the toxicity database for aminocyclopyrachlor and aminocyclopyrachlor-methyl have been reviewed. This includes a DER for a required acute neurotoxicity study.

II. ACTION REQUESTED

Please review and approve.

Rec'd in RC 11/6/2009 EV

III. BACKGROUND

Previously, a waiver request for an acute neurotoxicity study was submitted by the registrant. The Agency denied this waiver request (D361074). A waiver request for a 90-day dermal toxicity study was also received. The Agency granted this waiver and verified that the mutagenicity testing database was complete (D364576).

IV. ATTACHMENTS: SUMMARY TABLE

Guideline	MRID	Comments	File Name
None	47560018	New DER	47560018.DER
870.3100	47560008 47560009	New DER	47560008.DER
870.3100	47573403 47560007	New DER	47573403.DER
870.3100	47560010 47560011	New DER	47560010.DER
870.3150	47560012 47560013	New DER	47560012.DER
870.3200	47560014	New DER	47560014.DER
870.3250	Waived	Memo D364576	Not applicable
870.3700a	47560016	New DER	47560016.DER
870.3700b	47560015	New DER	47560015.DER
870.3800	57575101	New DER	57575101.DER
870.5100	47560033	New DER	47560033.DER
870.5100	47560019	New DER	47560019.DER
870.5300	47560020	New DER	47560020.DER
870.5375	47560021	New DER	47560021.DER
870.5395	47560022	New DER	47560022.DER
870.6200a	47725702	New DER	47725702.DER
870.6200b	47573403 47560007	New DER Same as 870.3100	47573403.DER
870.7485	47560023	New DER	47560023.DER
870.7485	47560024	New DER	47560024.DER
870.7800	47560025	New DER	47560025.DER
870.7800	47560026	New DER	47560026.DER

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR-METHYL (IN-KJM44)

Study Type: OPPTS Non-guideline; One-generation Reproduction Study in Rats

Work Assignment No. 6-1-211 A (MRID 47560018)

Prepared for
Health Effects Division
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Secondary Reviewer: Marquea D. King, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - [rat]; OPPTS None; OECD 415.

PC CODES: 288009 **DP BARCODE:** D361256
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor-methyl (99.7% a.i.)

SYNONYMS: IN-KJM44; Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylate

CITATION: Lewis, J.M. (2006). IN-KJM44: one-generation reproduction study in rats. E.I. du Pont de Nemours and Company, Haskell Laboratory for Health & Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-17315, May 19, 2006. MRID 47560018. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a non-guideline one-generation reproduction toxicity study (MRID 47560018), IN-KJM44 (Aminocyclopyrachlor-methyl; 99.7% a.i.; Lot # 027) was administered in the diet to 10 Sprague Dawley rats/sex/dose group at dietary levels of 0, 600, 5000, or 17,000 ppm (equivalent to an average of 0/0, 49/53, 398/435, and 1378/1489 mg/kg/day in P and F1 generation males/females during pre-mating). The P generation animals were fed the test diets for at least 28 days prior to mating to produce the F1 litters. Dams were allowed to deliver and rear their offspring until weaning on post-natal day (PND) 21. The F1 litters were culled to 4 pups/sex/litter (litter size permitting) on PND 4. In addition to the parameters typically evaluated in a reproductive toxicity study, hematology and clinical chemistry evaluations were performed on 5 rats/sex/dose from the P generation on Day 29 and at termination. Neurobehavioral evaluations (functional observational battery and motor activity measurements) were conducted on all P generation male (day 27) and female (day 28) animals during the pre-test period. At weaning, 10 F1 rats/sex/group were randomly selected to continue on study and were fed the same test diet concentration as their dam until termination at PND 60.

One P male was found dead on Day 6; however, this mortality was not considered treatment-related because no clinical signs of toxicity were observed in this animal prior to death, and no gross lesions were noted at necropsy. All other P and F1 males and females survived to scheduled termination. No treatment-related effects were observed on clinical signs, FOB, motor

activity, hematology, clinical chemistry, organ weights, gross pathology, or histopathology.

At 17,000 ppm, during the pre-mating period, food efficiency was decreased by 27% ($p<0.01$) for Days 0-7 in the P males compared to controls. In the P females at this dose, food consumption was decreased ($p<0.05$) by 11-15% for Days 0-7 and 7-14 and for the overall (Days 0-28) pre-mating period. However, body weights and body weight gains of the treated P females were unaffected by treatment. Body weights and body weight gains of the treated P males were comparable to controls throughout pre-mating, post-mating period (Days 28-60), and for the overall study (PND 0-60). In the P dams, body weights, body weight gains, food consumption, and food efficiency were unaffected by treatment throughout gestation and lactation.

In the F1 adults at 17,000 ppm, body weights were decreased by 8-17% ($p<0.05$) in the males from Day 0 and continuing through Day 21 and by 11-15% in the females on Days 0 and 7. Food consumption was decreased ($p<0.05$) by 9-17% in the males for Days 0-7, 14-21, 21-28, and 0-39 and by 8% in the females for Days 0-39.

The LOAEL for parental toxicity is 17,000 ppm (equivalent to 1378/1489 mg/kg/day in males/females), based on decreased food consumption and efficiency in the P generation and on decreased body weights and food consumption in the F1 generation. The NOAEL is 5000 ppm (equivalent to 398/435 mg/kg/day in males/females).

There were no effects of treatment on sex ratio or on gestation, live birth, viability, lactation, or litter survival indices during the post-natal period. Sexual maturation, organ weights, and gross pathology were unaffected by treatment.

At 17,000 ppm, eight pups from one litter were dehydrated compared to 0 controls. F1 pup body weights were decreased ($p<0.05$) by 9% on PND 14 and by 17% on PND 21 compared to controls. Pup body weight gains for the overall post-natal period (PND 0-21) were decreased (NS) by 20% compared to controls.

The LOAEL for offspring toxicity is 17,000 ppm (equivalent to 1378/1489 mg/kg/day in males/females), based on dehydration, decreased pup body weight gains, and decreased pup body weights late in lactation. The NOAEL is 5000 ppm (equivalent to 398/435 mg/kg/day in males/females).

There were no effects of treatment on mating or fertility indices, gestation duration, number of implantation sites, implantation efficiency, or post-implantation loss. Pre-coital duration was not reported, and sperm parameters were not measured. Estrus cycle stage for the pre-mating period was not reported. Microscopic examination of the vagina at study termination did not indicate any effects of treatment on the estrus cycle stage.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 17,000 ppm (equivalent to 1378/1489 mg/kg/day in males/females).

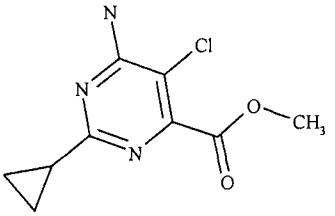
This study is classified as an **Acceptable/Non-guideline** one-generation reproduction study in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminocyclopyrachlor-methyl

Description:	Solid
Lot No.:	027
Purity:	99.7%
Compound stability:	Stable in the diet for up to 21 days when stored at room temperature or refrigerated
CAS # of TGAI:	858954-08-8
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley, Crl:CD®(SD)IGS BR
Age at study initiation:	Approximately 69 days
Body weight at study initiation:	308.4-380.2 g males 205.7-265.8 g females
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Rats were housed individually (except 1:1 during mating) in stainless steel, wire-mesh cages suspended above cage board during throughout the study for the males and until GD 20 for the females. Beginning on GD 20, mated females were housed in polycarbonate pans with bedding. Non-mated females were housed in polycarbonate pans with bedding beginning 7 days after the mating period.
Diet:	Certified Rodent LabDiet® 5002 (PMI Nutrition International, Inc., St. Louis, MO), *ad libitum*
Water:	Filtered (5 µm) tap water, *ad libitum*
Environmental conditions:	**Temperature** 18-26°C **Humidity** 30-70% **Air changes** Not provided **Light cycle** 12 hours light/12 hours dark
Acclimation period:	16 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: Start: April 28, 2005 End: approximately July 25, 2005

2. Mating procedure: Each female was continually housed on a one-to-one basis with a randomly selected, non-sibling male of the same dose group in the male's cage. Cohabitation was continued until evidence of copulation was observed or until two weeks had elapsed. Each female was examined for signs of mating daily, as evidenced by the presence of a vaginal plug or sperm in the vaginal smear. The day mating was confirmed was designated as gestation day (GD) 0. When copulation was confirmed, the female was returned to her home cage. The cohabitation period ended on the morning of Day 13 of co-housing.

3. **Study schedule:** The P generation animals were fed the test diets for at least 28 days prior to mating to produce the F1 litters. Females were allowed to deliver their litters, and the day on which delivery was complete was designated as lactation day (LD) 0. Litters were standardized to eight pups (4/sex when possible) by random culling of pups on post-natal day (PND) 4; litters of 8 pups or fewer were not culled. At weaning on PND 21, 10 offspring/sex/group (one pup/sex/litter where possible) were randomly selected to continue on study and were fed the same test diet concentration as their dam until termination on PND 60.

4. **Animal assignment:** The P animals were ranked by their most recently recorded body weight and randomly assigned to the test groups shown in Table 1. The randomization resulted in a distribution in which the mean body weights for all groups within a sex were not statistically different ($p>0.05$). On Day -1, two male rats (one control and one 600 ppm) were replaced because of observations found during the functional observational battery (FOB) performed on Day -3.

TABLE 1. Animal assignment[a]

Test group	Dose (ppm)[b]	Animals/group			
		P Males	P Females	F1 Males	F1 Females
Control	0	10	10	10	10
Low	600	10	10	10	10
Mid	5000	10	10	10	10
High	17,000	10	10	10	10

a Data were obtained from page 23 of the study report.
b Exposure to the test substance was continuous throughout the study.

5. **Dose-selection rationale:** It was stated that the doses used in the current study were selected by the Sponsor. No further information was provided.

6. **Test diet preparation and analysis:** For each dose level, an appropriate amount of the test substance was mixed directly with basal diet to yield the desired concentration. Control diets were mixed for a similar length of time. Test diets were prepared once per week and stored refrigerated until used. Prior to this study, stability of the test substance in the diet at 300 and 20,000 ppm were analyzed following 0, 7, 14, and 21 days at room temperature and 14 and 21 days refrigerated. Homogeneity and concentration analyses (mean of the homogeneity samples) were examined at each dose level in the current study from samples in the initial preparation and on Days 55 and 83. Additional concentration analyses were performed at each dose level on Day 111. All samples were taken in duplicate, with the exception of the concentration analyses from Day 111 for which a single sample was analyzed for each dose level.

Results

Homogeneity (% CV): 1-5%

Stability (% of Day 0): 91.0-92.5% after room temperature storage for 21 days
94.7-95.0% after refrigerated storage for 21 days

Concentration (% of nominal): 87.0-101.4%

The analytical data indicate that the mixing procedure was adequate and that the variation between the target and actual dosage to the study animals was acceptable.

7. **Dosage administration:** The test material was administered in the diet continuously throughout the study (i.e., P generation adults were fed the test diets *ad libitum* beginning 28 days prior to mating, and the selected F1 adults were fed the same test diet concentrations as their parents beginning on PND 22 until termination on PND 60).

C. PARENTAL/ADULT OBSERVATIONS

1. Observations

a. **Cage-side observations:** Animals were inspected twice daily for mortality and clinical signs of toxicity.

b. **Clinical examinations:** Detailed examinations were conducted once each week. Each rat was individually handled and examined for abnormal appearance and behavior in a standardized arena.

2. **Body weight and body weight gain:** In the P generation, body weights of the males were recorded prior to initiation of dosing, weekly throughout the study, and at termination. Body weight gains in these animals were reported for each weighing interval, and for the pre-mating period (Days 0-28), post-mating period (Days 28-60), and overall study (Days 0-60). P generation females were weighed prior to initiation of dosing, weekly throughout the pre-mating period, on GD 0, 7, 14, and 21, and on LD 0, 7, 14, and 21. Body weight gains were reported for each interval during pre-mating, gestation, and lactation, and for pre-mating Days 0-28, GD 0-21, and LD 0-21. In the F1 generation, body weights of the males and females were recorded weekly throughout the study beginning on Day 0 (PND 22) through termination on Day 39 (PND 60), and body weight gains were reported for each weighing interval and for Days 0-39 in these animals. Additionally, body weight was recorded in each F1 animal when it achieved sexual maturity (vaginal patency, preputial separation).

3. **Food consumption, food efficiency, and compound intake:** In the P generation, mean food consumption (g/animal/day) and food efficiency (body weight gain/food consumption) were reported weekly for both sexes during pre-mating and for Days 0-28; mean food consumption and efficiency in the females were also reported for GD 0-7, 7-14, 14-21, and 0-21, and for LD 0-7, 7-14, and 0-14. In the F1 generation, food consumption and food efficiency were reported for each weighing interval and for Days 0-39. Compound intake (mg/kg bw/day) values were calculated from the nominal dietary test material concentration, food consumption, and body weight data.

4. **Neurobehavioral assessment:** A neurobehavioral test battery, consisting of functional observational battery assessments (FOB) and motor activity (MA), was conducted on P males and females prior to test substance administration to obtain baseline measurements (Day -3 for P males and Day -2 for P females) and at the end of the pre-mating period (Day 27 in P males and Day 28 in P females). In order to accommodate the neurotoxicity testing facility, the FOB and MA assessments were conducted in 4 replicates over a 2-day period. Assignment to a given replicate was counterbalanced across all groups. The experimenter conducting the FOB and MA assessments was unaware of the treatment group designation of each animal.

a. **Functional observational battery:** Measurements/evaluations of P rats were performed in this order: inside the home cage; upon removal from the home cage and while being handled; and in a standard open field arena (approximately 85 x 59 x 20 cm). The scoring criteria for the FOB were provided in Appendix LL on pages 442-447 of the study report. The duration of the open field evaluation, environmental conditions, and further details concerning the conduct of the FOB were not provided. The following CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture	X	Ease of removal	X	Arousal
X	Palpebral closure	X	Ease of handling	X	Gait/coordination
X	Gait/coordination abnormalities	X	Muscle tone	X	Posture
X	Tremors	X	Fur/skin appearance	X	Respiration rate
X	Convulsions	X	Mucous membranes	X	Ease of respiration
		X	Dehydration	X	Number of rearing movements
	SENSORY OBSERVATIONS	X	Emaciation	X	Vocalizations
X	Approach response	X	Vocalizations	X	Convulsions
X	Touch response	X	Piloerection	X	Tremors
X	Startle response	X	Palpebral closure	X	Muscle spasms/ fasciculations
X	Pain response	X	Lacrimation	X	Diarrhea
X	Pupil response	X	Exophthalmus	X	Polyuria
		X	Salivation	X	Palpebral closure
			PHYSIOLOGICAL OBSER.		**NEUROMUSCULAR OBSER.**
			Body weight	X	Forelimb grip strength
		X	Body temperature	X	Hindlimb grip strength
				X	Landing foot splay

Fore- and hindlimb grip strength were measured by a strain gauge device (Chatillon® - Digital Force gauge), 3 trials per animal per session. Hindlimb splay was assessed by inking the hind paws and releasing the rat from a height of approximately 32 cm onto a piece of paper that covered a padded surface. Heel to heel distance was measured from the inked impressions and recorded. Rectal body temperature was recorded with an YSI Precision™ 4000 Thermometer and temperature probe. Following motor activity assessments (see below), pupillary constriction was measured immediately prior to removing the rats from the motor activity cages because the darkened room in which the apparatus is located facilitated observing the response. The presence or absence of pupillary constriction was assessed after

a beam of light was directed into each eye.

b. **Locomotor activity:** Immediately after completion of the FOB, the P rats were individually tested in one of 30 identical automated activity monitors (Coulbourn® Instruments). Groups were counterbalanced across the monitors and time of day to the fullest extent possible. The infrared monitoring device enabled measurement of two dependent variables, duration of movement and number of movements. A continuous movement, regardless of its duration, was counted as one movement. Each test session was 60 minutes in duration, and the results were expressed for the total session, as well as for 6 consecutive 10-minute blocks.

c. **Positive controls:** Positive control data were not provided with this study. Procedures and data describing the effects of trimethyltin, acrylamide, carbaryl, and d-amphetamine are presented in 5 separate reports (data not provided) [1, 2, 3, 4, 5]. The Sponsor stated that these positive control studies are the basis of certification of testing personnel, and that the data also establish that the equipment and procedures are capable of detecting effects that may be seen in neurotoxicity studies.

5. **Hematology and clinical chemistry:** On Day 29, after an overnight fast for at least 15 hours, blood samples were collected from the orbital sinus of 5 rats/sex/dose while under carbon dioxide anesthesia. Additional blood samples for coagulation parameters were collected from the abdominal *vena cava* at termination. Bone marrow smears and blood smears were prepared from all P animals surviving to scheduled termination; however, these smears were not examined. The following CHECKED (X) parameters were examined:

a. **Hematology**

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Red cell distribution width
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

[1] DuPont Haskell Laboratory (1995). Neurotoxicity evaluation of trimethyltin in rats (positive control study). Unpublished data, HLR 266-95.

[2] DuPont Haskell Laboratory (1996). Neurotoxicity evaluation of acrylamide in rats (positive control study). Unpublished data, HLR 293-95.

[3] DuPont Haskell Laboratory (1997). Neurotoxicity evaluation of carbaryl in rats (positive control study). Unpublished data, HLR-1997-00361.

[4] DuPont Haskell Laboratory (2000). Neurotoxicity evaluation of carbaryl in rats (positive control study). Unpublished data, DuPont-3468.

[5] DuPont Haskell Laboratory (1997). Neurotoxicity evaluation of amphetamine in rats (positive control study). Unpublished data, HL-1997-00686.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
	Magnesium	X	Urea nitrogen
X	Phosphorus	X	Total cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose (fasting)
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total protein
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Total bile acids
X	Alanine aminotransferase (ALT/also SGPT)		
X	Aspartate aminotransferase (AST/also SGOT)		
X	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

D. LITTER OBSERVATIONS: The following litter parameters (X) were recorded (Table 2):

TABLE 2. F1 Litter observations [a]

Observation	Time of observation (post-natal day)					
	Day 0	Day 4[b]	Day 4[c]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
Sex of each pup (M/F)	X	X	X	X	X	X
External alterations	X	X	X	X	X	X

a Data obtained from pages 27, 98, and 100 of the study report.
b Prior to standardization on PND 4
c After standardization on PND 4

At each examination period (PND 0, 4, 7, 14, and 21), litters were examined for mortality and abnormal behavior and appearance. F1 females designated for further evaluation were examined for vaginal patency daily beginning on PND 21 until achievement or PND 43; F1 males designated for further evaluation were examined for preputial separation beginning on PND 35 until achievement or PND 55. Body weights were recorded on the day of achievement.

E. POSTMORTEM OBSERVATIONS

1. **Parental animals:** At study termination, all surviving P and F1 rats were euthanized by exsanguination under carbon dioxide anesthesia and were subjected to a gross necropsy. Vaginal smears were collected from females on the day of necropsy, and estrus cycle stage determined. The uteri of all cohabited P females were examined for the presence and

number of implantation sites (method not provided). The following tissues (X) were collected for possible histological examination from all P and F1 adults, except for the thyroid gland and pancreas which were collected from the P females (not P males) and F1 males and females. The tissues were fixed in neutral buffered 10% formalin, except for the testis and epididymides which were fixed in Davidson's solution. Additionally, the (XX) organs were weighed (paired organs were weighed together).

	Males		Females		Both sexes
XX	Testes	XX	Ovaries [b]	XX	Pituitary [d]
XX	Epididymides	XX	Oviducts [b]	XX	Adrenals
XX	Prostate	XX	Uterus [c]	X	Thyroid
XX	Seminal vesicles [a]	XX	Cervix [c]	XX	Liver
XX	Coagulating glands [a]	X	Vagina	X	Pancreas [e]
				X	Gross lesions

a The seminal vesicles and coagulating glands were weighed together.
b The ovaries and oviducts were weighed together.
c The uterus and cervix were weighed together.
d Weighed after fixation
e The pancreas was collected from the P females (not males) and F1 males and females.

All tissues from the controls and high dose groups and all mated P adults that failed to produce a litter were examined microscopically. The severity of the microscopic findings was graded as minimal, mild, moderate, or severe.

2. **Offspring:** Pups that died during the lactation period were necropsied and discarded. Pups that were culled on PND 4 were euthanized by decapitation and discarded. F1 pups not selected on PND 21 to continue on study as adults (referred to as weanlings in the study report) were euthanized by exsanguination under carbon dioxide anesthesia and necropsied on PND 21. The thyroid gland, pancreas, and any gross lesions were collected from one weanling/sex/litter, preserved in 10% neutral buffered formalin, processed routinely, and subjected to gross and microscopic examination in the control and high dose groups. However, no organs were weighed.

F. DATA ANALYSIS

1. **Statistics:** The following statistical procedures were used. For litter parameters, the proportion of affected pups per litter or the litter mean were used as the experimental unit for statistical analyses. Significance was denoted at $p<0.05$. The statistical methods were considered appropriate.

Parameter	Statistical procedure		
	Preliminary tests	Preliminary test is not significant	Preliminary test is significant
Body weight and body weight gain Food consumption and food efficiency, Gestation duration Number of implantation sites Implantation efficiency Number of pups/litter Live born, viability, and lactation indices Sexual maturation Clinical pathology [a] Organ weights	Levine's test for homogeneity of variances and Shapiro-Wilk test for normality [b]	One-way analysis of variance (ANOVA) and Dunnett's test	Kruskal-Wallis test and Dunn's test
Clinical signs Mating, fertility, gestation, and litter survival indices FOB descriptive parameters	None	Cochran-Armitage test for trend [c]	
Sex ratio (covariate: litter size) Pup weights (covariates: litter size, sex ratio)	Levine's test for homogeneity and Shapiro-Wilk test for normality [d]	Analysis of covariance and Dunnett-Hsu	Non-parametric analysis of covariance
Motor activity [e] Grip strength Foot splay Rearing Body temperature	Levine's test for homogeneity of variances and Shapiro-Wilk test for normality [d]	Repeated measures ANOVA followed by Linear contrasts	Sequential application of the Jonckheere-Terpstra trend test

a When an individual observation was recorded as being less than the limit of determination, calculations were performed on half the recorded value. For example, if bilirubin was reported as < 0.1, then 0.05 was used for any calculations performed with that data. When an individual observation was recorded as being greater than the limit of determination, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, then 1.083 was used for any calculations performed with that data. Significance was denoted at $p\leq0.05$ or $p\leq0.01$.

b If the Shapiro-Wilk test was not significant but Levine's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was used, followed by Dunn's test if necessary.

c If there was significant non-monotonicity in the dose-response, then Fisher's Exact Test with a Bonferroni correction was used.

d A transformation may have been applied as needed to achieve a normal distribution and homogeneous variances.

e Test day and 10-minute interval will be used as repeated-measure factors.

2. Indices

Reproductive indices: The following reproductive indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Mating index (%) = # copulated / # cohabited x 100

Fertility index (%) = # pregnant / # copulated x 100

Gestation index (%) = # litters with at least one live pup / # litters x 100

Birth index (Implantation efficiency, %) = # pups born / # implantation sites x 100

Additionally, post-implantation loss was calculated by the reviewers as:

Post-implantation loss (%) = 100 – birth index

Offspring viability indices: The following offspring indices were calculated by the performing laboratory from lactation records of litters in the study:

Live birth index (%) = # pups born alive / # pups born x 100

Viability index (%) = # pups alive on PND 4 (pre-cull) / # pups born alive x 100

Lactation index (%) = # pups alive on PND 21 / # pups alive on PND 4 (post-cull)

Litter survival (%) = # litters weaned / # viable litters delivered

3. **Historical control data:** Historical control data were not provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** One 17,000 ppm P male was found dead on Day 6; however, this mortality was not considered treatment-related because no clinical signs of toxicity were observed in this animal prior to death, and no gross lesions were noted at necropsy. All other P animals and all F1 males and females survived to scheduled termination.

b. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed. The most common observation was hair loss, observed in all groups in both males and females of both generations.

2. Body weight, body weight gain, food consumption, and food efficiency

a. **Pre-mating:** At 17,000 ppm, during the pre-mating period, food efficiency was decreased by 27% ($p<0.01$) for Days 0-7 in the P males compared to controls (Table 3a). In the P females at this dose, food consumption was decreased ($p<0.05$) by 11-15% for Days 0-7 and 7-14 and for the overall (Days 0-28) pre-mating period. The investigators reported that body weight gain for the overall pre-mating period was decreased by 35% in the 17,000 ppm females compared to controls, and that body weights at the end of the pre-mating period were 6% lower than controls. However, the reviewers disagree with the Sponsor that the reported decreases in body weights and weight gains were due to treatment because they were not dose-related, not statistically significant, and because the decrease in body weight

at the end of pre-mating was minor. Body weights and body weight gains of the treated P males were comparable to controls throughout pre-mating, post-mating period (Days 28-60), and for the overall study (PND 0-60).

In the F1 adults at 17,000 ppm, body weights were decreased by 8-17% ($p<0.05$) in the males from Day 0 and continuing through Day 21 and by 11-15% in the females on Days 0 and 7. Food consumption was decreased ($p<0.05$) by 9-17% in the males for Days 0-7, 14-21, 21-28, and 0-39 and by 8% in the females for Days 0-39.

There were no other treatment-related findings in body weights, body weight gains, food consumption, or food efficiency. The decreased (↓9-17%; $p<0.05$) food consumption at 5000 ppm in the F1 males for Days 0-7 and 14-21 and in the F1 females beginning on Day 21 were considered unrelated to treatment because they did not result in decreased body weights or body weight gains and were generally unrelated to dose.

TABLE 3a. Selected mean (±SD) body weights (g), body weight gains (g), and food consumption (g/rat/day) during pre-mating[a]

Observation/study day [b]		Dose Group (ppm)			
		0	600	5000	17,000
P Males (n=9, 17000 ppm; n=10 all others)					
Body weight	Day 0	344.1 ± 18.6	339.5 ± 12.5	336.9 ± 22.4	338.7 ± 12.8
	Day 28	449.6 ± 25.6	441.3 ± 37.8	445.1 ± 39.1	435.1 ± 36.5
	Day 60	530.7 ± 31.8	519.7 ± 40.2	518.5 ± 50.7	517.7 ± 52.1
Body weight gain	Days 0-28	105.4 ± 11.6	101.8 ± 29.2	108.2 ± 20.4	96.1 ± 28.4
	Day 28-60	81.1 ± 11.9	78.4 ± 17.8	73.4 ± 14.7	82.6 ± 18.0
	Days 0-60	186.5 ± 16.7	180.2 ± 31.8	181.6 ± 33.2	178.7 ± 44.1
Food consumption	Days 0-28	25.9 ± 1.5	25.9 ± 3.8	24.9 ± 1.7	25.0 ± 2.0
Food efficiency	Days 0-7	0.220 ± 0.017	0.157 ± 0.120	0.201 ± 0.037	0.161 ± 0.065** (↓27)
	Days 0-28	0.145 ± 0.011	0.138 ± 0.026	0.155 ± 0.020	0.135 ± 0.031
P Females (n=10)					
Body weight	Day 0	233.8 ± 11.9	232.4 ± 12.0	231.4 ± 15.6	231.0 ± 15.1
	Day 28	270.3 ± 14.0	261.6 ± 26.1	265.5 ± 28.4	254.8 ± 25.1
Body weight gain	Days 0-28	36.6 ± 5.6	29.1 ± 15.5	34.1 ± 15.9	23.8 ± 15.2
Food consumption	Days 0-7	19.7 ± 1.4	19.5 ± 1.5	18.2 ± 1.7	17.4 ± 2.3* (↓12)
	Days 7-14	20.2 ± 1.2	19.1 ± 2.1	18.4 ± 2.6	17.2 ± 1.7* (↓15)
	Days 0-28	19.3 ± 1.0	18.5 ± 1.6	18.1 ± 2.0	17.2 ± 1.8* (↓11)
Food efficiency	Days 0-28	0.068 ± 0.010	0.055 ± 0.025	0.065 ± 0.026	0.047 ± 0.027
F1 Males (n=9-10)					
Body weight	Day 0	62.3 ± 3.5	59.6 ± 3.7	58.0 ± 5.3	51.7 ± 3.3* (↓17)
	Day 21	235.4 ± 11.3	235.5 ± 17.6	221.1 ± 22.9	216.2 ± 14.0* (↓8)
	Day 39	396.5 ± 22.7	395.4 ± 37.1	371.2 ± 34.8	364.6 ± 19.3
Body weight gain	Days 0-39	334.2 ± 23.4	335.8 ± 34.7	313.1 ± 32.3	312.9 ± 17.8
Food consumption	Days 0-7	13.8 ± 0.8	13.2 ± 1.0	11.5 ± 2.8** (↓17)	11.4 ± 1.3** (↓17)
	Day 14-21	24.5 ± 1.5	23.6 ± 1.9	22.0 ± 2.0* (↓10)	22.2 ± 2.7* (↓9)
	Day 21-28	27.1 ± 2.3	26.6 ± 4.5	25.0 ± 1.7	23.7 ± 2.8* (↓13)
	Day 0-39	23.4 ± 1.5	23.6 ± 2.1	21.9 ± 1.4	21.4 ± 1.3* (↓9)
Food efficiency	Days 0-39	0.369 ± 0.019	0.366 ± 0.018	0.366 ± 0.020	0.375 ± 0.009
F1 Females (n=9-10)					
Body weight	Day 0	58.3 ± 2.4	55.7 ± 3.4	54.7 ± 4.5	49.6 ± 4.1* (↓15)
	Day 7	95.3 ± 4.5	94.3 ± 5.9	92.8 ± 7.8	84.8 ± 6.5** (↓11)
	Day 39	227.0 ± 22.4	223.3 ± 25.9	207.3 ± 23.1	217.8 ± 28.8
Body weight gain	Day 0-39	168.7 ± 23.8	167.6 ± 26.7	152.6 ± 21.8	168.3 ± 27.8
Food consumption	Days 21-28	19.0 ± 1.6	18.2 ± 1.5	16.8 ± 1.6** (↓12)	17.5 ± 2.2
	Day 28-35	19.5 ± 2.5	19.4 ± 2.1	17.1 ± 1.8** (↓12)	18.1 ± 2.2
	Day 35-39	20.5 ± 2.4	19.5 ± 3.3	17.6 ± 1.8* (↓14)	18.3 ± 2.1
	Day 0-39	17.3 ± 1.0	16.8 ± 1.2	15.7 ± 1.0** (↓9)	15.9 ± 1.6** (↓8)
Food efficiency	Days 0-39	0.246 ± 0.024	0.255 ± 0.024	0.249 ± 0.024	0.264 ± 0.021

a Data were obtained from Tables 17 through 20, 25 through 32, and 37 through 40 on pages 72-76, 81-88, 93-96 of the study report.

b. Gestation: Body weights, body weight gains, food consumption, and food efficiency in the treated P dams were comparable to controls throughout gestation (Table 3b).

TABLE 3b. Mean (±SD) body weights (g), body weight gains (g), and food consumption (g/rat/day) during gestation[a]

Observation/gestation day		Dose Group (ppm) 0	600	5000	17,000
P Females (n=10)					
Body weight	GD 0	277.6 ± 24.2	267.3 ± 25.2	270.4 ± 27.5	258.5 ± 25.3
	GD 7	320.3 ± 20.8	312.9 ± 25.0	314.7 ± 28.5	301.2 ± 22.8
	GD 14	356.9 ± 25.1	348.7 ± 24.0	348.5 ± 34.0	331.7 ± 26.3
	GD 21	447.3 ± 25.3	445.5 ± 22.3	436.1 ± 38.5	420.1 ± 31.6
Body weight gain	GD 0-21	169.7 ± 22.8	180.1 ± 17.2	165.7 ± 17.8	161.7 ± 22.8
Food consumption	GD 0-21	26.3 ± 2.3	26.9 ± 1.7	24.9 ± 2.5	24.3 ± 1.9
Food efficiency	GD 0-21	0.308 ± 0.043	0.319 ± 0.024	0.319 ± 0.033	0.317 ± 0.032

a Data were obtained from Tables 21, 22, 33, and 34 on pages 77, 78, 89, and 90 of the study report.

c. **Lactation:** In the P generation, body weights, body weight gains, food consumption, and food efficiency were unaffected by treatment throughout lactation (Table 3c). Body weight loss was observed in all groups during the final week of lactation (LD 14-21); this loss was significantly ($p<0.05$) less severe in the 17,000 ppm group (-0.9 g) compared to controls (-25.6 g). Similarly, body weight gains for the overall lactation period (LD 0-21) were significantly higher ($p\leq0.05$) in this group (26.4 g) compared to controls (0.7 g). Although the investigators described this finding as "less than expected weight loss" and linked this finding to the subsequently observed decreased ($p<0.05$) pup weights during the PND 14 and 21, the reviewers do not consider a significantly less severe weight loss in the dams to be adverse.

TABLE 3c. Mean (±SD) body weights (g), body weight gains (g), and food consumption (g/rat/day) during lactation[a]

Observation/lactation day		Dose Group (ppm) 0	600	5000	17,000
P Females (n=10)					
Body weight	LD 0	337.9 ± 19.7	331.3 ± 22.7	321.7 ± 28.3	313.0 ± 28.5
	LD 7	347.2 ± 13.5	338.9 ± 24.5	343.2 ± 20.9	319.4 ± 42.4
	LD 14	364.2 ± 17.9	357.5 ± 17.1	352.3 ± 23.1	340.3 ± 29.0
	LD 21	338.6 ± 24.6	338.6 ± 13.1	337.4 ± 27.1	339.4 ± 22.8
Body weight gain	LD 14-21	-25.6 ± 15.1	-18.9 ± 11.9	-14.9 ± 12.8	-0.9 ± 16.2*
	LD 0-21	0.7 ± 17.2	7.2 ± 20.1	15.8 ± 14.7	26.4 ± 12.7*
Food consumption	LD 0-14	47.2 ± 5.0	44.8 ± 4.1	47.0 ± 9.2	45.5 ± 4.9
Food efficiency	LD 0-14	0.041 ± 0.023	0.042 ± 0.027	0.047 ± 0.019	0.044 ± 0.030

a Data were obtained from Tables 23, 24, 35, and 36 on pages 79, 80, 91, and 92 of the study report.

3. **Test substance intake:** The mean test substance intake for both generations during pre-mating is considered to be representative of the achieved intake for the entire study (Table 4).

TABLE 4. Mean test substance intake (mg/kg/day in males/females) during pre-mating [a]				
Generation	Dose (ppm)			
	0	600	5000	17,000
P generation	0/0	38.0/43.9	304/352	1059/1190
F1 generation	0/0	60.1/62.8	491/518	1697/1787
Mean [b]	**0/0**	**49/53**	**398/435**	**1378/1489**

a Data were obtained from Tables 1, 2, 5, and 6 on pages 56, 57, 60, and 61 of the study report.
b Calculated by the reviewers as the average of the P and F1 generations separately for each sex.

4. Reproductive function

a. Estrus cycle duration and periodicity: Estrus cycle stage for the pre-mating period was not reported. Microscopic examination of the vagina at study termination did not indicate any effects of treatment on the estrus cycle stage.

b. Sperm parameters: Not measured.

5. Reproductive performance: Reproductive performance data are presented in Table 5. There were no effects of treatment on mating or fertility indices, gestation duration, number of implantation sites, implantation efficiency, or post-implantation loss. Pre-coital interval data were not reported.

TABLE 5. Reproductive performance [a]				
Parameter	Dose Group (ppm)			
	0	600	5000	17,000
P generation				
Mating index (%)	100.0	100.0	100.0	100.0
Fertility index (%)	100.0	90.0	100.0	100.0
Gestation duration (days)	22.0	21.8	22.1	22.0
Number of implantation sites	14.9 ± 2.2	14.9 ± 1.5	14.6 ± 2.0	14.2 ± 2.3
Implantation efficiency (%) [b]	94.5 ± 7.5	97.2 ± 6.4	96.2 ± 4.1	97.5 ± 4.3
Post-implantation loss (%) [c]	5.5	2.8	3.8	2.5

a Data were obtained from Table 41 on page 97 of the study report.
b Implantation efficiency (%) = # pups born/ # implantation sites x 100
c Post-implantation loss (%, calculated by the reviewers) = 100% - implantation efficiency

6. Neurobehavioral assessment

a. Functional observational battery (FOB): There were no effects of treatment on any FOB parameters in either sex.

b. Motor activity: There were no effects of treatment on the duration of movement or the number of movements in either sex. In the 17,000 ppm females, the number of movements

were decreased ($p<0.05$) by 29% compared to controls during the 6th 10-minute interval (Table 6). However, a comparable mean and standard deviation (78 ± 55) were noted in this group for the baseline measurements prior to initiation of dosing. Furthermore, the mean number of movements for the total 60-minute session in this group was not significantly lower than controls. Therefore, this decrease was considered incidental.

TABLE 6. Mean (±SD) number of movements [a]

Consecutive 10-min Interval	Dose Group (ppm)			
	0	600	5000	17,000
P males (n=10)				
1	133 ± 23	133 ± 21	130 ± 17	134 ± 14
2	131 ± 33	131 ± 21	135 ± 18	135 ± 15
3	94 ± 44	118 ± 29	124 ± 19	106 ± 34
4	65 ± 41	85 ± 55	62 ± 46	88 ± 57
5	53 ± 52	62 ± 53	22 ± 30	69 ± 59
6	25 ± 26	45 ± 45	12 ± 16	30 ± 38
Total 60-minute session	501 ± 155	574 ± 156	484 ± 119	561 ± 166
P females (n=10)				
1	129 ± 24	140 ± 19	145 ± 31	127 ± 22
2	110 ± 16	117 ± 37	131 ± 30	109 ± 30
3	105 ± 31	120 ± 28	113 ± 26	104 ± 37
4	83 ± 40	87 ± 34	93 ± 46	74 ± 42
5	99 ± 37	69 ± 49	88 ± 43	75 ± 46
6	111 ± 41	96 ± 44	87 ± 37	79 ± 44* (↓29)
Total 60-minute session	637 ± 112	629 ± 140	656 ± 103	566 ± 130

a Data were obtained from Tables 52 and 53 on pages 123 and 124 of the study report.
* Significantly different from the controls at $p<0.05$

7. Blood analyses

a. Hematology: There were no effects of treatment on any hematology parameters in either sex.

b. Clinical chemistry: There were no effects of treatment on clinical chemistry. In the 17,000 ppm females, bilirubin was decreased by 20% ($p<0.05$) compared to controls. Although an increase in this parameter may indicate liver toxicity, the observed decrease is considered spurious. The treated values for all other clinical chemistry parameters were comparable to controls.

8. Parental postmortem results

a. Organ weights: There were no effects of treatment on organ weights in either generation. In the P females, absolute and relative (to body weight) ovary weights were decreased by 19-20% ($p<0.05$) compared to controls. However, no gross or microscopic findings were observed in the ovaries, and reproductive success was not compromised in these animals. Absolute prostate weights were decreased by 22% ($p<0.05$) in the 5000 ppm P males;

however, this finding was unrelated to dose. All other organ weights in the treated P males and in the F1 males and females were comparable to controls.

b. **Pathology**

1) **Macroscopic examination:** There were no treatment-related gross lesions in the P generation or in the F1 adults or weanlings.

2) **Microscopic examination:** There were no treatment-related microscopic findings. In the F1 weanlings, decreased zymogen in the acinar cells of the pancreas was noted in 2/10 males at 17,000 ppm compared to 0/10 controls. However, because the incidences of this finding were mild in severity and were not noted in the P generation or in the F1 adults, they were considered incidental to treatment.

B. OFFSPRING

1. **Viability and clinical signs:** Litter parameters are presented in Table 7. There were no effects of treatment on sex ratio or on gestation, live birth, viability, lactation, or litter survival indices. At 17,000 ppm, eight pups from one litter were dehydrated compared to 0 controls. No other clinical signs were noted.

TABLE 7. Litter parameters [a]

Parameter	Dose Group (ppm)			
	0	600	5000	17,000
F1 generation				
Mean # pups /litter				
Born	14.4	14.4	14.1	14.0
Born alive	14.1	14.4	14.1	13.8
PND 4 pre-cull	13.8	14.4	13.8	13.7
PND 4 post-cull	8.00	8.00	8.00	8.00
PND 7	8.00	7.9	7.9	8.00
PND 14	8.00	7.9	7.9	8.00
PND 21	8.00	7.9	7.9	8.00
Sex ratio (% males)	0.55	0.54	0.47	0.48
Gestation index (%)	100.0	100.0	100.0	100.0
Live birth index (%)	97.7	100.0	100.0	98.8
Viability index (%)	97.7	100.0	98.0	99.3
Lactation index (%)	100.0	98.6	98.8	100.0
Litter survival index (%)	100.0	100.0	100.0	100.0

a Data were obtained from Table 42 on page 98 of the study report.

2. **Body weight:** Pup body weight and body weight gain data are presented in Table 8. At 17,000 ppm, F1 pup body weights were decreased ($p<0.05$) by 9% on PND 14 and by 17% on PND 21 compared to controls. Pup body weight gains for the overall post-natal period (PND 0-21) were decreased by 20% compared to controls. There were no treatment-related effects on body weights or body weight gains in the F1 pups at 600 or 5000 ppm.

TABLE 8. Mean (±SD) pup weights and body weight gains (g) [a]				
Post-natal day (PND)	Dose Group (ppm)			
	0	600	5000	17,000
F1 pups				
PND 0	6.5 ± 0.5	6.6 ± 0.4	6.7 ± 0.4	6.7 ± 0.5
4 (pre-cull)	11.1 ± 1.2	10.9 ± 0.8	11.0 ± 1.1	10.9 ± 0.9
4 (post-cull)	11.0 ± 1.2	10.9 ± 0.7	11.0 ± 1.1	10.9 ± 1.0
7	18.0 ± 1.7	17.9 ± 1.4	17.6 ± 1.6	16.9 ± 1.9
14	37.2 ± 2.8	36.0 ± 2.1	36.0 ± 2.6	33.9 ± 3.0* (↓9)
21	60.3 ± 3.7	57.1 ± 3.6	56.5 ± 4.6	50.0 ± 4.1* (↓17)
PND 0-21 [b]	53.8	50.5	49.8	43.3 (↓20)

a Data were obtained from Table44 on page 100 of the study report.
b Calculated by reviewers from data presented in this table.
* Significantly different from controls; $p<0.05$

3. **Developmental landmarks:** There were no treatment-related effects on achievement of preputial separation or vaginal patency in the F1 offspring. The mean age at pre-putial separation was significantly higher ($p<0.05$) at 600 ppm (43.9 days) compared to controls (42.1 days); however, this delay in sexual maturation was minor and unrelated to dose.

4. **Offspring postmortem results**

a) **Organ weights:** There were no effects of treatment on offspring organ weights.

b) **Pathology**

1) **Macroscopic examination:** There were no treatment-related macroscopic findings in the F1 pups.

2) **Microscopic examination:** Not examined.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** It was concluded that the NOAEL for reproductive toxicity was 17,000 ppm (the highest dose tested), and the LOAEL was not observed. The LOAEL for parental toxicity was 17,000 ppm based on decreased body weight and food consumption in the P females during pre-mating (followed by lower than anticipated weight loss during lactation) and on decreased body weights in the F1 adults. The LOAEL for offspring toxicity was 17,000 ppm based on decreased pup weights during lactation, continuing with recovery during the post-weaning period. There were no adverse effects observed during clinical pathology or during neurobehavioral evaluations in the P rats; and organ weights and gross and microscopic pathological findings were not affected by treatment at any dietary concentration.

B. REVIEWER COMMENTS

1. **PARENTAL ANIMALS:** One P male was found dead on Day 6; however, this mortality was not considered treatment-related because no clinical signs of toxicity were observed in this animal prior to death, and no gross lesions were noted at necropsy. All other P and F1 males and females survived to scheduled termination. No treatment-related effects were observed on clinical signs, FOB, motor activity, hematology, clinical chemistry, organ weights, gross pathology, or histopathology.

   At 17,000 ppm, during the pre-mating period, food efficiency was decreased by 27% ($p<0.01$) for Days 0-7 in the P males compared to controls. In the P females at this dose, food consumption was decreased ($p<0.05$) by 11-15% for Days 0-7 and 7-14 and for the overall (Days 0-28) pre-mating period. The investigators reported that body weight gain for the overall pre-mating period was decreased by 35% in the 17,000 ppm females compared to controls, and that body weights at the end of the pre-mating period were 6% lower than controls. However, the reviewers disagree with the Sponsor that the reported decreases in body weights and weight gains were due to treatment because they were not dose-related, not statistically significant, and because the decrease in body weight at the end of pre-mating was minor. Body weights and body weight gains of the treated P males were comparable to controls throughout pre-mating, post-mating period (Days 28-60), and for the overall study (PND 0-60).

   In the F1 adults at 17,000 ppm, body weights were decreased by 8-17% ($p<0.05$) in the males from Day 0 and continuing through Day 21 and by 11-15% in the females on Days 0 and 7. Food consumption was decreased ($p<0.05$) by 9-17% in the males for Days 0-7, 14-21, 21-28, and 0-39 and by 8% in the females for Days 0-39.

   The LOAEL for parental toxicity is 17,000 ppm (equivalent to 1378/1489 mg/kg/day in males/females), based on decreased food consumption and efficiency in the P generation and on decreased body weights and food consumption in the F1 generation. The NOAEL is 5000 ppm (equivalent to 398/435 mg/kg/day in males/females).

2. **OFFSPRING:** There were no effects of treatment on sex ratio or on gestation, live birth, viability, lactation, or litter survival indices during the post-natal period. Sexual maturation, organ weights, and gross pathology were unaffected by treatment.

   At 17,000 ppm, eight pups from one litter were dehydrated compared to 0 controls. F1 pup body weights were decreased ($p<0.05$) by 9% on PND 14 and by 17% on PND 21 compared to controls. Pup body weight gains for the overall post-natal period (PND 0-21) were decreased by 20% compared to controls.

   The LOAEL for offspring toxicity is 17,000 ppm (equivalent to 1378/1489 mg/kg/day in males/females), based on dehydration and decreased pup body weight gains, and decreased pup body weights late in lactation. The NOAEL is 5000 ppm (equivalent to 398/435 mg/kg/day in males/females).

3. **REPRODUCTIVE TOXICITY:** There were no effects of treatment on mating or fertility indices, gestation duration, number of implantation sites, implantation efficiency, or post-implantation loss. Pre-coital duration was not reported, and sperm parameters were not measured. Estrus cycle stage for the pre-mating period was not reported. Microscopic examination of the vagina at study termination did not indicate any effects of treatment on the estrus cycle stage.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 17,000 ppm (equivalent to 1378/1489 mg/kg/day in males/females).

There were no adverse effects observed during clinical pathology or during neurobehavioral evaluations in the P rats; and organ weights and gross and microscopic pathological findings were not affected by treatment at any dietary concentration.

This study is classified as an **acceptable/non-guideline** one-generation reproduction study in rats.

C. **STUDY DEFICIENCIES:** The following minor study deficiencies were noted but do not affect the conclusions or acceptability of this DER:

- Historical control data were not provided.
- Although citations for positive control data were provided, no data were reported.
- In Volume 2 of the study report, the labels for Appendices KK, LL, MM, NN, OO, and PP are used twice but contain different data. This is because the second set of data is the FOB data.

DATA EVALUATION RECORD

DPX-KJM44 (AMINOCYCLOPYRACHLOR-METHYL)

Study Type: OPPTS 870.3100 [§82-1a], Subchronic Oral Toxicity Study in Rats

Work Assignment No. 5-1-209 A (MRIDs 47560008 and 47560009)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity in Rats [feeding]; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 288009 **DP BARCODE:** D361256
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor methyl (95% a.i.)

SYNONYMS: DPX-KJM44; Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylate

CITATION: Anand, S.S. (2008) DPX-KJM44 Technical: subchronic toxicity 90-day feeding study in rats. E.I. du Pont de Nemours and Company, Newark, DE. Laboratory Project ID: DuPont-22570, July 2, 2008. MRID 47560008. Unpublished.

Mawn, M.P. (2008) DPX-KJM44 Technical: subchronic toxicity 90-day feeding study in rats (supplement 1). E.I. du Pont de Nemours and Company, Newark, DE. Laboratory Project ID: DuPont-21491, August 7, 2008. MRID 47560009. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRIDs 47560008 and 47560009), Aminocyclopyrachlor methyl (95% a.i.; Lot #068) was administered to 10 Sprague-Dawley rats/sex/dose group in the diet at dose levels of 0, 600, 2000, 6000, or 18,000 ppm (equivalent to 0/0, 35/43, 117/133, 347/405, and 1022/1219 mg/kg/day in males/females) for approximately 90 days (94 or 95 days in males and 95 or 96 days in females). On Days 60 and 87, blood was collected from the orbital sinus or tail vein of 5 rats/sex/dose group for the determination of plasma concentration of the test substance (methyl aminocyclopyrachlor) and the metabolites DPX-MAT28 (aminocyclopyrachlor) and IN-LXT69.

All animals survived to scheduled sacrifice. There were no treatment-related clinical signs or effects on ophthalmology. Functional Observational Battery (FOB) parameters were measured in a different study (MIRD 47560018). At 18,000 ppm, body weight decreased with time and was decreased (↓8%) in males and females by Day 91, although this decrease was not statistically significant. Statistically significant ($p<0.05$) decreases in body weight were observed in females

at earlier time points (Day 42 and Day 84). Body weight gain also decreased with time, and was significantly decreased over the treatment period (Day 0-91) in males (↓14%) and females (↓24%). Food consumption also tended to decrease with time and was significantly decreased in males (↓9%) and females (↓11%) over the treatment period (Day 0-Day 91). Food efficiency was significantly decreased in females (↓31%) from Days 0-7. Food efficiency over the treatment period (Days 0-91) was decreased in males (↓5%) and females (↓15%), but these decreases were not statistically significant.

There were no adverse effects of treatment on hematology, clinical chemistry, or urinalysis parameters.

There were decreases in organ weights that were not considered adverse due to lack of corroborating gross and histopathological changes. Absolute and relative (to brain weight) liver weights were decreased in the 18,000 ppm males (↓13%) and absolute, relative (to brain weight), and relative (to body weight) heart weights in the 6000 and 18,000 ppm females were decreased (↓8-16%).

The parent compound DPX-KJM44 (aminocyclopyrachlor methyl) was not detected in any of the plasma samples. DPX-MAT28 was the predominant plasma metabolite. This metabolite was dose-dependently increased in all treated groups, with plasma concentrations of 254, 1489, 6444, and 20,660 ng/mL in the males and 376, 2202, 5316, and 29,784 ng/mL in the females fed the 600, 2000, 6000, and 18,000 ppm diets respectively. Plasma concentrations of the metabolite IN-LXT69 were considerably lower than DPX-MAT28, but were also dose-dependently increased in all treated groups, with plasma concentrations of 12, 45, 136, and 439 ng/mL in the males and 15, 45, 125, and 415 ng/mL in the females fed the 600, 2000, 6000, and 18,000 ppm diets respectively. All of the control plasma samples were below the limit of quantitation (LOQ) for DPX-KJM44 and IN-LXT69. Plasma concentrations for DPX-MAT28 were below the LOQ in all of the control samples for the males and in two of the samples from the females; however, 3 of the 5 female plasma samples had DPX-MAT28 at concentrations 2 to 4 times the LOQ.

The LOAEL is 18,000 ppm (equivalent to 1022/1219 mg/kg/day in males/females) based on decreased body weights, body weight gains, food consumption, and food efficiency in both sexes. The NOAEL is 6000 ppm (equivalent to 347/405 mg/kg/day in males/females).

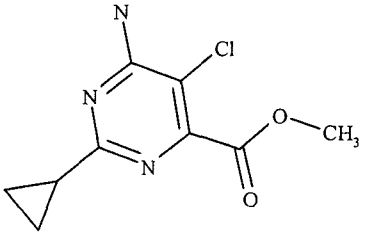
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminocyclopyrachlor methyl

Description:	Solid
Lot #:	068
Purity:	95% aminocyclopyrachlor methyl (89% aminocyclopyrachlor acid equivalents on a calculated basis)
Stability:	Stable in the diet for up to 14 days when stored at room temperature or 21 days when stored refrigerated
CAS # of TGAI:	858954-83-3
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley, Crl:CD(SD)
Age/weight at study initiation:	Approximately 52 days old/221.0-253.3 g males and 157.8-198.4 g females
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Individually in stainless steel, wire mesh cages suspended above cage boards.
Diet:	Certified Rodent LabDiet #5002®, (PMI Nutrition International, LLC, St. Louis, MO), *ad libitum*; except overnight prior to blood collection
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air changes:	Not reported
Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	8 days

B. STUDY DESIGN

1. In-life dates: Start: August 24, 2007 End: November 28, 2007

2. Animal assignment: Rats were selected for use on study based on adequate body weight gain and freedom from any clinical signs of disease or injury or ophthalmology abnormalities. They were distributed by computerized, stratified randomization into the study groups presented in Table 1 so that there were no statistically significant differences among group body weight means within a sex. The weight variation of selected rats did not exceed ±20% of the mean weight for each sex.

TABLE 1. Study design [a]			
Test group	**Dietary concentration in M/F (ppm) [b]**	**Achieved intake in M/F (mg/kg/day)**	**# Males/Females**
Control	0	0/0	10/10
Low	600	35/43	10/10
Mid-low	2000	117/133	10/10
Mid-high	6000	347/405	10/10
High	18,000	1022/1219	10/10

a Data were obtained from page 13 and from Tables 9 and 10 on pages 54 and 56 of MRID 47560008.
b Dietary concentrations (w/w) were adjusted for the sponsor-supplied purity (95% a.i.)

3. **Dose-selection rationale:** A previous 28-day study in rats was conducted with IN-KJM44 at dietary concentrations of 0, 600, 2000, 6000, and 20,000 ppm (Dupont-17313). The following summary was presented in the study report, however, no data were provided. There were no treatment-related effects on survival, clinical signs, ophthalmological observations, clinical pathology parameters, food consumption, organ weights, or gross pathology. Body weights, body weight gains, and food efficiency were decreased in the 20,000 ppm males and females. Additionally at 20,000 ppm, the males had thyroid follicular cell hypertrophy and pancreatic acinar cell apoptosis and the females had induction of hepatic β-oxidation activity. Furthermore, no reproductive toxicity was noted in a one-generation reproduction feeding study (Dupont-17315) in which rats were fed test diets up to 17,000 ppm. No maternal or developmental toxicity was observed in a developmental toxicity pilot study in rats dosed up to 1000 mg/kg/day via gavage from gestation day (GD) 6-21. Based on these findings, the dietary concentrations presented in Table 1 were selected for the current study. The 18,000 ppm concentration was expected to produce a limit dose (1000 mg/kg/day) and possible systemic toxicity, but was not expected to cause mortality. Dietary concentrations of 2000 or 6000 ppm were expected to produce systemic toxicity or be the NOAEL.

4. **Treatment preparation, administration, and analysis:** It was stated that the test diets were prepared by adding the appropriate amount of the test material (adjusted for purity) directly to the basal diet and thoroughly mixing for a period of time that, by experience or pretest determination, was adequate to ensure homogeneous distribution in the diet. Control diets were mixed for the same period of time. Test diets were prepared every other week and refrigerated until used. Test diets stored beyond 21 days after preparation were not fed to the study animals. From the initial diet preparation, duplicate samples were taken from the top, middle, and bottom of the mixer for each dietary concentration for analysis of concentration and homogeneity of the test material in the diets. Concentration analyses for this preparation were reported as the average of the top, middle, and bottom of these samples. Additionally, the concentration of each dietary level was verified near the middle and end of the study. It was stated that, in the previously conducted one generation reproductive toxicity study (Dupont-17315), stability of the test material in the diet was confirmed at 300 and 20,000 ppm for up to 14 days at room temperature and 21 days in refrigerated storage. However, no data were provided.

Results

Homogeneity (% CV): 1-3%

Concentration (% nominal): 84.1-119.1%

The analytical data provided indicate that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was marginally acceptable.

5. **Statistics:** Body weights, body weight gains, food consumption, food efficiency, clinical pathology, and organ weight data were subjected to statistical analyses. These data were first analyzed using Levene's test for homogeneity of variances and Shapiro-Wilk test for normal distribution. If the results of Levene's test and Shapiro-Wilk test were not significant ($p \geq 0.05$), indicating homogeneous variances and normally distributed data, significant differences among groups were measured using one-way analysis of variance (ANOVA) followed by pair-wise comparisons of the treated groups with controls using Dunnett's test. If the Shapiro-Wilk test was not significant (i.e., data were normally distributed) but Levene's test was significant (i.e., variances were not homogeneous), a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, significant differences among groups were measured using Kruskal-Wallis test followed by pair-wise comparisons of the treated groups with controls using Dunn's test. When an individual observation was recorded as being less than the limit of determination, calculations were performed on half the recorded value. For example, if bilirubin was reported as < 0.1, then 0.05 was used for any calculations performed with that data. When an individual observation was recorded as being greater than the limit of determination, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, then 1.083 was used for any calculations performed with that data. Significance was denoted at $p \leq 0.05$ or $p \leq 0.01$. The statistical methods were considered appropriate.

C. METHODS

1. Observations

a. **Cage-side observations:** Cage-side observations for mortality, moribundity, and clinical signs of toxicity were performed each morning at approximately the same time each day (±2 hours) and additionally in the afternoon.

b. **Clinical examinations:** At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. Detailed clinical observations were conducted on each rat in a standardized arena. These observations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, and presence of clonic, tonic, stereotypical, or bizarre behavior.

c. **Neurobehavioral evaluations:** It was stated that the neurobehavioral effects were examined in the previously conducted one generation reproductive toxicity study (MRID 47560018, Dupont-17315) using functional observational battery and motor activity assessments; and therefore, these evaluations were not conducted in the current study.

2. **Body weight:** All animals were weighed prior to study initiation, on Day 0, weekly throughout the study, and at termination. Body weight gains were reported for each weekly interval throughout the study and for the overall (Days 0-91) study.

3. **Food consumption, food efficiency, and compound intake:** The amount of food consumed by each rat was measured between weighing intervals, and the mean daily food consumption (g/animal/day) was determined and reported for each weekly interval and for the overall study. Using the food consumption and body weight data, food efficiency (g body weight gain/g food consumption) and test substance intake (mg/kg bw/day) were calculated and were reported for these intervals. Overall test substance intake is reported in Table 1 of this DER.

4. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were conducted on all animals pre-test (Day -7) and prior to termination (Day 84). Both eyes of each rat were examined by focal illumination and indirect ophthalmoscopy. The eyes were examined in subdued light after mydriasis was produced.

5. **Hematology and clinical chemistry:** After an overnight fast for at least 15 hours, blood samples were collected from the orbital sinus of each of the rats while under carbon dioxide anesthesia on Days 94-95 for males and Days 95-96 for females. Additional blood samples for coagulation parameters were collected from the abdominal vena cava at termination. Bone marrow smears and blood smears were prepared from each animal surviving to scheduled termination; however, these smears were not examined. The following CHECKED (X) parameters were examined:

a. **Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Red cell distribution width
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose (fasting)*
	ENZYMES (more than 2 hepatic enzymes)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Plasma concentrations:** On Day 60, a blood sample (approximately 0.5 mL) was collected from the orbital sinus or tail vein of each rat for the determination of the test substance (methyl aminocyclopyrachlor) and the metabolites DPX-MAT28 (aminocyclopyrachlor) and IN-LXT69. However, initial results from the control samples taken on Day 60 revealed quantifiable levels of DPX-MAT28 in 8 of the 10 samples. It was believed that this contamination occurred during sample collection, thus, additional blood samples were collected on Day 87. The blood samples were processed to plasma and analyzed using high performance liquid chromatography with detection by tandem mass spectrometry (LC/MS/MS). The low level calibration standard had an average peak-to-peak response greater than 10x the average noise for control plasma samples. The lower limit of quantitation (LOQ) of the method was calculated by multiplying the low level calibration standard concentration times the minimum sample preparation dilution factor of 20x. The QC fortification recoveries ranged from 94-101%. This range demonstrates acceptable analytical method performance.

7. **Urinalysis*:** On the day prior to termination, each rat was placed in a metabolism cage and fasted after 3 p.m. for at least 15 hours, during which time urine was collected. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose (fasting)
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Optional for 90-day oral rodent studies

8. **Sacrifice and pathology:** Following blood sampling at study termination, all animals were euthanized by exsanguination under carbon dioxide anesthesia and subjected to a gross necropsy. The order of sacrifice for scheduled deaths was stratified across groups. The following CHECKED (X) tissues from all animals were collected. Additionally, the (XX) organs were weighed. Paired organs were weighed together.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow* [a]	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (and optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone (sternum and/or femur) [b]
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Peyer's patches [c]
X	Lungs*	X	Mammary gland (females)*		
X	Nose*	X	Vagina		
X	Pharynx*	X	Cervix		
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
a Bone marrow was collected with the femur and sternum.
b The sternum and femur with knee joint were collected.
c Peyer's patches were collected with the intestines.

Testes, epididymides, and eyes were fixed in modified Davidson's solution. All other tissues were fixed in 10% neutral buffered formalin. All tissues collected from the control and high dose groups were processed routinely, stained with hematoxylin and eosin, and evaluated microscopically. In addition, all gross lesions were examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All animals survived until scheduled termination.

2. **Clinical signs:** There were no treatment-related clinical signs. At 18,000 ppm, black discharge was noted in the eye(s) of 2/10 males compared to 0 controls on 5 occasions between Days 56-70. Additionally in the females at this dose, one rat was hyper-reactive on 3 occasions during Days 63-77, and high posture was observed in 2/10 rats on 8 occasions during Days 56-91. However, these clinical observations were considered unrelated to treatment because they were transient and minimal in incidence. All other clinical observations were unrelated to dose.

B. **BODY WEIGHTS AND WEIGHT GAINS:** Selected body weight and body weight gain data are presented in Table 2. At 18,000 ppm, body weights were decreased by 3-9% (not significant [NS]) compared to controls throughout treatment in the males. In the females at this dose, body weights were decreased throughout treatment, with decreases of 2-8% (NS) from Days 7-35 and on Day 91 and significant ($p<0.05$) decreases of 9-11% from Days 42-84. Body weight gains for the overall (Days 0-91) study were decreased ($p<0.05$) by 14% in the males and by 24% in the females. There were no effects of treatment on body weights or body weight gains at 600, 2000, or 6000 ppm in either sex. The only other significant difference from controls was a decrease of 17% ($p<0.05$) in body weight gains for the 600 ppm males for Days 49-56, which was unrelated to dose.

TABLE 2. Mean (±SD) body weights and body weight gains (g) in rats fed DPX-KJM44 in the diet for up to 91 days. [a]

Parameter/ Time interval		Dose (ppm) 0	600	2000	6000	18,000
Males						
Body weight	Day 0	236.3 ± 8.6	236.8 ± 8.9	237.2 ± 8.8	236.5 ± 9.2	237.3 ± 9.1
Body weight	Day 7	291.0 ± 15.6	291.1 ± 13.2	292.4 ± 16.7	286.5 ± 16.2	281.8 ± 10.7 (↓3)
Body weight	Day 70	541.1 ± 43.1	531.0 ± 39.5	531.3 ± 58.7	535.7 ± 35.7	499.2 ± 35.9 (↓8)
Body weight	Day 91	572.4 ± 45.9	560.3 ± 38.8	558.0 ± 63.8	562.7 ± 35.2	526.4 ± 37.5 (↓8)
Body weight gain	Days 63-70	17.4 ± 3.9	19.0 ± 6.6	17.8 ± 5.1	14.8 ± 3.5	12.1 ± 3.4*(↓30)
Body weight gain	Days 0-91	336.1 ± 40.3	323.5 ± 33.2	320.9 ± 58.3	326.1 ± 32.9	289.1 ± 32.6* (↓14)
Females						
Body weight	Day 0	174.5 ± 9.8	175.8 ± 10.9	175.6 ± 6.3	173.9 ± 7.1	178.6 ± 10.7
Body weight	Day 7	200.5 ± 10.4	198.9 ± 7.7	198.9 ± 9.1	193.1 ± 10.5	195.5 ± 12.4 (↓2)
Body weight	Day 42	253.5 ± 17.5	253.8 ± 18.0	248.8 ± 17.3	239.2 ± 20.3	229.5 ± 16.8* (↓9)
Body weight	Day 49	261.6 ± 18.4	265.2 ± 20.7	261.3 ± 18.8	244.5 ± 21.9	234.2 ± 19.0* (↓10)
Body weight	Day 56	269.0 ± 19.6	272.8 ± 21.6	266.1 ± 16.9	249.0 ± 17.0	245.2± 19.0*(↓9)
Body weight	Day 70	276.7 ± 22.2	283.5± 24.3	277.0 ± 16.0	259.8 ± 21.3	249.2 ± 16.6*(↓10)
Body weight	Day 77	282.9 ± 21.5	289.1 ± 24.1	283.5 ± 16.6	262.5 ± 22.4	255.8 ± 18.4*(↓10)
Body weight	Day 84	288.1 ± 23.9	294.4 ± 23.8	288.5 ± 16.1	263.3 ± 22.4	257.5 ± 16.3* (↓11)
Body weight	Day 91	287.9 ± 26.8	292.8 ± 25.1	288.4 ± 16.2	270.2 ± 22.5	264.4 ± 19.5 (↓8)
Body weight gain	Days 0-7	26.0 ± 4.9	23.1 ± 8.7	23.3 ± 5.3	19.2 ± 9.5	16.9 ± 4.9*(↓35)
Body weight gain	Days 0-91	113.4 ± 23.5	117.0 ± 27.7	112.8 ± 15.6	96.3 ± 21.3	85.7 ± 14.9* (↓24)

a Data were obtained from Tables 1 through 4 on pages 37-44 of MRID 47560008; n=10.
* Significant at $p<0.05$.

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE:

1. **Food consumption and food efficiency:** Selected food consumption and food efficiency data are presented in Table 3. In the 18,000 ppm males, food consumption was decreased by

3-15% throughout treatment compared to controls, with a decrease of 9% ($p<0.05$) for the overall (Days 0-91) study. A minor decrease of 5% (NS) was noted in food efficiency for Days 0-91 in the 18,000 ppm males. In the females, food consumption was decreased by 4-13% at 6000 ppm and by 5-16% at 18,000 ppm throughout the study compared to controls, resulting in decreases ($p<0.05$) of 9 and 11% in the 6000 and 18,000 ppm groups, respectively. Food efficiency for Days 0-91 was decreased by 7% and 15% (NS) in the females at 6000 and 18,000 ppm, respectively. Although the decreases in food consumption and food efficiency in the 6000 ppm females were likely due to treatment, they were not considered adverse because they did not affect body weights or body weight gains at this dose. There were no other treatment-related effects on food consumption or food efficiency.

TABLE 3. Mean (±SD) food consumption (g/animal/day) and food efficiency (g body weight gain/g food consumed) in rats fed DPX-KJM44 in the diet for up to 91 days. [a]

Parameter/ Time interval	Dose (ppm) 0	600	2000	6000	18,000
Males					
Food consumption Days 14-21	24.8 ± 2.3	25.8 ± 1.4	25.8 ± 2.8	25.5 ± 2.3	24.0 ± 1.2 (↓3)
Food consumption Days 63-70	27.4 ± 2.0	26.7 ± 2.4	27.1 ± 2.9	26.3 ± 1.8	23.3 ± 2.5* (↓15)
Food consumption Days 0-91	26.2 ± 1.9	25.9 ± 1.5	26.0 ± 2.3	25.8 ± 1.5	23.8 ± 1.4* (↓9)
Food efficiency Days 0-91	0.140 ± 0.008	0.137 ± 0.010	0.135 ± 0.013	0.139 ± 0.008	0.133 ± 0.011 (↓5)
Females					
Food consumption Days 0-7	16.7 ± 1.2	16.8 ± 1.6	15.5 ± 0.9	15.3 ± 1.7 (↓8)	15.9 ± 2.6 (↓5)
Food consumption Days 28-35	17.7 ± 1.9	17.6 ± 1.9	16.2 ± 1.4	16.6 ± 2.4 (↓6)	14.9 ± 1.6* (↓16)
Food consumption Days 77-84	18.5 ± 2.3	18.9 ± 1.7	17.2 ± 1.3	16.1 ± 1.9* (↓13)	15.8 ± 1.1* (↓15)
Food consumption Days 84-91	16.6 ± 2.2	17.1 ± 1.9	16.0 ± 1.1	15.9 ± 1.8 (↓4)	15.0 ± 1.2 (↓10)
Food efficiency Days 0-7	0.221 ± 0.038	0.195 ± 0.062	0.215 ± 0.045	0.174 ± 0.075	0.153 ± 0.039* (↓31)
Food consumption Days 0-91	17.6 ± 1.3	18.0 ± 1.5	16.6 ± 0.8	16.0 ± 1.6* (↓9)	15.7 ± 0.9* (↓11)
Food efficiency Days 0-91	0.071 ± 0.012	0.071 ± 0.011	0.075 ± 0.009	0.066 ± 0.010 (↓7)	0.060 ± 0.009 (↓15)

a Data were obtained from Tables 5 through 8 on pages 45-52 of MRID 47560008; n=10.
* Significant at $p<0.05$.

2. **Compound intake:** The mean daily achieved intake (mg/kg bw/day) for Days 0-91 for each sex is included in Table 1 of this DER.

D. OPHTHALMOSCOPIC EXAMINATION: No ocular abnormalities were found in any of the animals examined on Day 84.

E. BLOOD ANALYSES

1. **Hematology:** There were no effects of treatment on hematology. The only significant ($p<0.05$) difference from controls was a decrease of 39% in the number of neutrophils in the 6000 ppm females. However, this difference was unrelated to dose.

2. **Clinical chemistry:** In the males, total protein was decreased by 4-7% ($p<0.05$) in all treated groups compared to controls. Additionally in the 18,000 ppm males, decreases ($p<0.05$) were observed in albumin (↓3%) and globulin (↓8%). However, these minor decreases were not considered adverse. No other dose-related differences from controls were found.

3. **Plasma concentrations:** The parent compound DPX-KJM44 (aminocyclopyrachlor methyl) was not detected in any of the plasma samples (Table 4). DPX-MAT28 was the predominant plasma metabolite. This metabolite was dose-dependently increased in all treated groups, with plasma concentrations of 254, 1489, 6444, and 20,660 ng/mL in the males and 376, 2202, 5316, and 29,784 ng/mL in the females fed the 600, 2000, 6000, and 18,000 ppm diets respectively. Plasma concentrations of the metabolite IN-LXT69 were considerably lower than DPX-MAT28, but were also dose-dependently increased in all treated groups, with plasma concentrations of 12, 45, 136, and 439 ng/mL in the males and 15, 45, 125, and 415 ng/mL in the females fed the 600, 2000, 6000, and 18,000 ppm diets respectively. All of the control plasma samples were below the limit of quantitation (LOQ) for DPX-KJM44 and IN-LXT69. Plasma concentrations for DPX-MAT28 were below the LOQ in all of the control samples for the males and in two of the samples from the females; however, 3 of the 5 female plasma samples had DPX-MAT28 at concentrations 2 to 4 times the LOQ.

TABLE 4. Mean (±SD) plasma concentration (ng/mL) of DPX-KJM44 (aminocyclopyrachlor methyl) and the metabolites DPX-MAT28 (aminocyclopyrachlor) and IN-LXT69 in rats fed in the diet for 87 days. [a]

Parameter	Dose (ppm)				
	0	600	2000	6000	18,000
Males					
DPX-KJM44	<10.0	<10.0	<10.0	<10.0	<10.0
DPX-MAT28	<10.0	254 ± 60	1489 ± 805	6444 ± 2568	20660 ± 7669
IN-LXT69	<10.0	12.28 ± 2	45.3 ± 6	136.4 ± 22.8	438.8 ± 57.9
Females					
DPX-KJM44	<10.0	<10.0	<10.0	<10.0	<10.0
DPX-MAT28	26 ± 11	376 ± 148	2202 ± 775	5316 ± 2935	29784 ± 17872
IN-LXT69	<10.0	15.04 ± 0.9	45.3 ± 9.4	125.0 ± 17.2	415.4 ± 63.6

a Data were obtained from Table 2 on page 18 of MRID 47560009; n=5.

F. URINALYSIS: There were no effects of treatment on any urinalysis parameter in either sex.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** The following organ weights were significantly decreased ($p<0.05$) compared to controls: (i) absolute and relative (to brain weight) liver weights in the 18,000 ppm males (↓13%); (ii) relative (to brain weight) kidney weights in the 6000 and 18,000 ppm males (↓11-12%); and (iii) absolute, relative (to brain weight), and relative (to body weight) heart weights in the 6000 and 18,000 ppm females (↓8-16%). Relative (body weight) heart weights were also decreased by 9% ($p<0.05$) in the 2000 ppm females.

2. **Gross pathology:** No treatment-related findings in gross pathology were found at necropsy. Thick cecum was noted at 18,000 ppm in one male (#510) and in one female (#552); however, this finding was minimal in incidence. Incidences of all other gross lesions also were unrelated to dose and only occurred in a single animal per dose group.

3. **Microscopic pathology:** There were no treatment-related findings in histopathology. In the

cecum from the 18,000 ppm males, mucosal inflammation was noted in 3/10 rats (male #509, male #510, female #551) and hyperplasia in 1/10 rats (male #510) compared to controls. Chronic progressive nephropathy was found in 2/10 females (#554, #558) at this dose compared to controls). In addition to being minor in incidence, the severity of each of these findings was minimal or mild. Incidences of all other microscopic findings were unrelated to dose.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS:

The investigators concluded that the LOAEL was 18,000 ppm (equivalent to 1022 and 1219 mg/kg/day in males and females, respectively) based on adverse effects on body weight and food intake parameters.

B. REVIEWER'S COMMENTS:

All animals survived to scheduled sacrifice. There were no deaths or clinical signs of toxicity and no treatment-related effects on ophthalmoscopy, hematology, clinical chemistry, urinalysis, gross pathology, or histopathology. FOB parameters were measured in a different study (MIRD 47560018).

At 18,000 ppm, body weights were decreased by 3-9% (NS) compared to controls throughout treatment in the males. In the females at this dose, body weights were decreased throughout treatment, with decreases of 2-8% (NS) from Days 7-35 and on Day 91 and significant ($p<0.05$) decreases of 9-11% from Days 42-84. Body weight gains for the overall (Days 0-91) study were decreased ($p<0.05$) by 14% in the males and by 24% in the females. There were no effects of treatment on body weights or body weight gains at 600, 2000, or 6000 ppm in either sex.

In the 18,000 ppm males, food consumption was decreased by 3-15% throughout treatment compared to controls, with a decrease of 9% ($p<0.05$) for the overall study. A minor decrease of 5% (NS) was noted in food efficiency for Days 0-91 in the 18,000 ppm males. In the females, food consumption was decreased by 4-13% at 6000 ppm and by 5-16% at 18,000 ppm throughout the study compared to controls, resulting in decreases ($p<0.05$) of 9 and 11% in the 6000 and 18,000 ppm groups, respectively. Food efficiency for Days 0-91 was decreased by 7% and 15% (NS) in the females at 6000 and 18,000 ppm, respectively. Although the decreases in food consumption and food efficiency in the 6000 ppm females were likely due to treatment, they were not considered adverse because they did not affect body weights or body weight gains at this dose.

There were no adverse effects of treatment on hematology, clinical chemistry, or urinalysis parameters.

Thick cecum was noted at 18,000 ppm in 1/10 male and 1/10 females. However, corroborative microscopic evidence of mucosal inflammation and hyperplasia was found only for the male, and there were examples of mucosal inflammation in the absence of thickened

cecum or other gross pathological findings. There were no other gross findings with microscopic correlates, and all observed gross and macroscopic findings were not considered adverse.

Absolute and relative (to brain weight) liver weights were decreased in the 18,000 ppm males (↓13%) and absolute, relative (to brain weight), and relative (to body weight) heart weights in the 6000 and 18,000 ppm females were decreased (↓8-16%). These changes were not considered adverse due to lack of corroborating gross and histopathological changes.

The parent compound DPX-KJM44 (aminocyclopyrachlor methyl) was not detected in any of the plasma samples. DPX-MAT28 was the predominant plasma metabolite. This metabolite was dose-dependently increased in all treated groups, with plasma concentrations of 254, 1489, 6444, and 20,660 ng/mL in the males and 376, 2202, 5316, and 29,784 ng/mL in the females fed the 600, 2000, 6000, and 18,000 ppm diets respectively. Plasma concentrations of the metabolite IN-LXT69 were considerably lower than DPX-MAT28, but were also dose-dependently increased in all treated groups, with plasma concentrations of 12, 45, 136, and 439 ng/mL in the males and 15, 45, 125, and 415 ng/mL in the females fed the 600, 2000, 6000, and 18,000 ppm diets respectively. All of the control plasma samples were below the limit of quantitation (LOQ) for DPX-KJM44 and IN-LXT69. Plasma concentrations for DPX-MAT28 were below the LOQ in all of the control samples for the males and in two of the samples from the females; however, 3 of the 5 female plasma samples had DPX-MAT28 at concentrations 2 to 4 times the LOQ.

The LOAEL is 18,000 ppm (equivalent to 1022/1219 mg/kg/day in males/females) based on decreased body weights, body weight gains, liver and heart weights (absolute and relative), food consumption, and food efficiency in both sexes. The NOAEL is 6000 ppm (equivalent to 347/405 mg/kg/day in males/females).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in rats.\

C. **STUDY DEFICIENCIES:** The following deficiency was noted but does not affect the conclusions of this DER or the acceptability of the study:

- It was stated that the test material was shown to be stable in the diet for up to 14 days at room temperature or 21 days in refrigerated storage, and a citation for a previously conducted one-generation reproduction toxicity study was provided. However, no data were available for review.

DATA EVALUATION RECORD

AMINOCYLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.3100 [§82-1a]; 90-Day Oral Toxicity-feeding-rats and OPPTS 870.6200b [§82-7]; Subchronic Neurotoxicity Study in Rats

Work Assignment No. 5-1-209 B (MRID 47573403) 2nd (MRID 47560007)

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity-feeding-rats, OPPTS 870.3100 [§82-1a] (rodent); OECD 408 and Subchronic neurotoxicity study in rats (dietary), OPPTS 870.6200b [§82-7]; OECD 424.

PC CODE: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Anand, S.S. (2008) DPX-MAT28 Technical: subchronic toxicity 90-day feeding study in rats. E.I. du Pont de Nemours and Company, Newark, DE. Laboratory Project ID: DuPont-21490, July 2, 2008. MRID 47573403. Unpublished.

Anand, S.S. (2008). DPX-MAT28 Technical: subchronic toxicity 90-day feeding study in rats. Supplement I. E.I. du Pont de Nemours and Company, Newark, DE. Laboratory Project ID: DuPont-21490, August 21 2008. MRID 47560007. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a subchronic oral and neurotoxicity study (MRID 47573403), aminocyclopyrachlor (DPX-MAT28; 92.2% a.i.; Batch No. DPX-MAT28-009) was administered in the diet to Crl:CD(SD) rats (15/sex/dose) at doses (adjusted for purity) of 0, 600, 2000, 6000, or 18,000 ppm (equivalent to 34.8/44.8, 114.3/145.7, 349.4/448.0, and 1044.6/1424.9 mg/kg/day) for 13 weeks (94-95 days in males and 96-97 days in females). Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed prior to treatment and during Weeks 4, 8, and 13 on 10 rats/sex/group. On Day 56, plasma concentrations of DPX-MAT28 and the metabolite IN-LXT69 were measured in 5/sex/dose. At study termination, gross and histopathology were performed on 10 rats/sex/group, while 5 rats/sex/group were anesthetized and perfused *in situ* for neuropathological examination. The

tissues from the perfused animals in the control and 18,000 ppm groups were subjected to histopathological evaluation of brain, spinal cord, and peripheral nervous system tissues.

All animals survived to the day of scheduled sacrifice. One control male and one 6000 ppm female were accidentally killed during blood collection just prior to necropsy.

No treatment-related effects were observed in daily or weekly clinical observations and there were no test substance-related opthamological signs for either sex. There were no test substance-related or statistically significant effects on functional observational battery (FOB) parameters: forelimb and hindlimb grip strength, footsplay, body temperature, or behavioral parameters in either sex. Additionally, there were no test substance-related or statistically significant effects on duration of movement or number of movements measured in the motor activity analysis of males and females.

At 18,000 ppm, decreases ($p \leq 0.05$; except as noted) in body weights were observed in the males from Days 28-91 (↓6-9%, NS at Days 56 and 70) and in the females on Days 56, 70, and 77 (↓8-10%). Overall (Days 0-91) body weight gain was decreased ($p \leq 0.05$) by 15-18% in both sexes. Significant decreases ($p \leq 0.05$) in food consumption were observed at 18,000 ppm during Days 0-7, 21-28, and 42-49 in males (↓7-8%) and during Days 70-77 in females (↓11%). Decreased ($p \leq 0.05$) food efficiency was noted in males during Days 21-28, 70-77, and 77-84 (↓18-73%) and in females during Days 62-70 (↓112%). Overall (Days 0-91) food efficiency was decreased ($p \leq 0.05$) by 11% in males and 17% in females.

There were no adverse effects of the test substance on organ weights, ophthalmology, hematology, coagulation, clinical chemistry, or urinalysis parameters in males and females. There were no effects of the test substance on brain weights or gross or microscopic neuropathology in either sex at any dose.

The plasma concentrations of DPX-MAT28 and the metabolite IN-LXT69 were measured on Day 56 by high performance liquid chromatography with detection by tandem mass spectrometry (LC/MS/MS, MRID 47560007). DPX-MAT28 was the most abundant metabolite in male and female rats, with the highest concentrations at 10, 268 and 13, 428 ng/mL for 18,000 ppm male and female rats, respectively. In contrast, plasma levels of IN-LXT68 were lower for this dose group at concentrations of 82 and 80 ng/mL for males and females, respectively. The average concentration of DPX-MAT29 in plasma was higher in females than in males at all dose levels. The IN-LXT69 plasma concentrations were below the limit of quantitation for the 600 ppm dietary concentration. The average IN-LXT69 plasma concentrations appeared to be similar for males and females. There was no apparent plateau for the DPX-MAT28 of IN-LXT68 plasma concentrations when compared to dietary intake levels (e.g. plasma concentrations increased with increasing concentration in the diet).

The LOAEL for systemic effects is 18,000 ppm (1044.6/1424.9 mg/kg/day), based on decreased body weights, body weight gains, food consumption, and food efficiency in both sexes. The NOAEL is 6000 ppm (349.4/448.0 mg/kg/day).

The LOAEL for neurotoxicity was not observed. The NOAEL is 18,000 ppm (1044.6/1424.9 mg/kg/day) in both sexes.

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) and a subchronic neurotoxicity study (OPPTS 870.6200b; OECD 424) in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28

Description: White solid

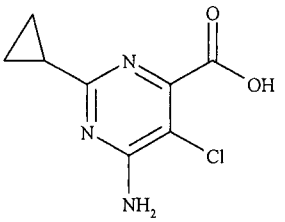
Lot No.: DPX-MAT28-009

Purity (w/w): 92.2% a.i.

Stability of compound: Stable in the diet for up to 14 days when stored at room temperature or 21 days when stored refrigerated

CAS #: 858956-08-8

Structure:



2. Vehicle: Diet

3. Test animals

Species: Rat

Strain: Sprague Dawley (Crl:CD[SD])

Age at study initiation: Approximately 51 days

Mean weight at study initiation: 253-256 g males
174-180 g females

Source: Charles River Laboratories, Inc. (Raleigh, NC)

Housing: Rats were housed individually in suspended, stainless steel, wire-mesh cages.

Diet: Certified Rodent LabDiet® 5002 (PMI Nutrition International, LLC, St. Louis, MO), *ad libitum* except when fasted

Water: Tap water, *ad libitum*

Environmental conditions

- **Temperature:** 18-26°C
- **Humidity:** 30-70%
- **Air changes:** Not provided
- **Photoperiod:** 12 hours light/12 hours dark

Acclimation period: Approximately 20 days

B. STUDY DESIGN

1. In life dates: Start: February 2, 2007; End: May 10, 2008

2. Animal assignment/dose levels: The animals were distributed by computerized, stratified randomization into study groups (Table 1) so that there were no statistically significant

differences among group body weight means within a sex. The weight variation of selected rats did not exceed ±20% of the mean weight for each sex.

TABLE 1. Study design [a]

Test group	Dietary concentration in M/F (ppm)	Compound intake (mg/kg/day in M/F)	No. rats/sex: Main Study	Satellite groups [b]
			# M/F	# M/F
Control	0	0/0	10	5
Low	600	34.8/44.8	10	5
Mid	2000	114.3/145.7	10	5
Mid-high	6000	349.4/448.0	10	5
High	18,000	1044.6/1424.9	10	5

a Data were obtained from pages 14,17, and 59-62 of MRID 47573403.
b On Day 56, blood was collected from 5 mice/sex/group for evaluation of plasma concentrations of the parent compound and/or its metabolites.

3. **Dose-selection rationale:** Based upon the results of a 28-day toxicity (feeding) study in rats[1] (data not reported in MRID 47573403), the doses shown in Table 1 were selected for the present study. In the range-finding study, the Sponsor stated that rats were treated at 0, 600, 2000, 6000, and 20,000 ppm. Decreased body weight, body weight gain, and food efficiency occurred at 20,000 ppm in both sexes. In the 20,000 ppm males, thyroid follicular cell hypertrophy and pancreatic acinar cell apoptosis were noted. In the 20,000 ppm females, an induction of hepatic β-oxidation activity was observed. No treatment-related effect was reported at 6000 ppm and lower.

4. **Dose preparation and analysis:** For each dose level, an appropriate amount of the test substance (adjusted for purity) was mixed directly with basal diet to yield the desired concentration. Control diets were mixed for a similar length of time. Test diets were prepared every 7-11 days and stored refrigerated until used. Homogeneity (top, middle, bottom) was verified in all dose levels of the first preparation. Stability was determined in dietary formulations from the initial batch containing 300 and 18,000 ppm of the test compound following room temperature storage for up to 14 days or refrigerated storage for up to 21 days. Samples for concentration verification were taken from all dose levels on Days 48 and 83, and the average concentration from the homogeneity measurements were also considered. In each case, duplicate sample of the dietary preparation were taken, and samples that were not analyzed immediately were frozen.

Results

Homogeneity (% CV): 3-7%

[1] DuPont Haskell (2006). IN-KJM44: Repeated-dose oral toxicity 28-day feeding study in rats. Unpublished report, DuPont-17313.

Stability (% of nominal): 91.1-108.3% after room temperature storage for 14 days
94.4-118.0% after refrigerated storage for 21 days

Concentration (% of nominal): 82.8-103.0%

Dose (ppm)	Range (% of nominal)
600	88.8-103.0
2000	86.5-101.0
6000	82.8-95.0
18,000	86.1-95.0

The analytical data indicate that the mixing procedure was marginally adequate and that the variation between the target and actual dosage to the study animals was marginally acceptable. It is desirable to have homogeneity of ≤5% and a concentration of ±10% nominal.

5. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure		
	Preliminary tests	**Preliminary test is not significant**	**Preliminary test is significant**
Body weight Body weight gain Food consumption Food efficiency Organ weight	Levine's test for homogeneity and Shapiro-Wilk test for normality [a]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Incidence of FOB (descriptive parameters)	None	Cochran-Armitage test for trend [b]	
Motor activity [c] Grip strength Footsplay Body temperature	Levine's test for homogeneity and Shapiro-Wilk test for normality	Repeated measures analysis of variance followed by linear contrasts	A normalizing, variance stabilizing transformation or non-parametric test (Dunn's) or sequential application of the Jonckheere-Terpstra trend test

a If the Shapiro-Wilk test was not significant but Levine's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was used, followed by Dunn's test if necessary.
b If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact Test with a Bonferroni correction was used.
c Test day and 10-minute interval were used as repeated-measure factors.

Statistical significance was denoted at $p \leq 0.05$. The statistical methods were considered appropriate.

C. METHODS

1. Clinical Observations:

a. Cageside observations: Animals were inspected daily for signs of toxicity and mortality.

b. Clinical examinations: General clinical examinations were conducted daily at approximately the same time each day (± 2 hours), except on days when neurological evaluations were

performed. Acute clinical signs of toxicity were not recorded on day 96 for some females. This was inadvertent, only happened on one day, and was not believed to affect the interpretation of the study.

c. **Neurological evaluations:** The following evaluations (measurements) were performed once each week. Each rat was individually handled and examined for abnormal appearance and behavior in a standardized arena. The detailed clinical observations included (but were not limited to) evaluation of fur, skin , eyes, mucus membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical, or bizarre behavior.

2. **Body weight and body weight gain:** All animals were weighed prior to treatment, weekly until scheduled termination, and on the day of sacrifice. Body weight gains were reported for each interval between body weight measurements and also for Days 0-91.

3. **Food consumption, food efficiency, and compound intake:** Food consumption (g/animal/day) and food efficiency (body weight gain/food consumption) were reported weekly and for Days 0-91. Compound intake (mg/kg bw/day) values were calculated from the nominal dietary test material concentration, food consumption, and body weight data.

4. **Ophthalmology Evaluation:** Ophthalmology evaluations were first conducted by a veterinary ophthalmologist pre-test (Day -7). Animals with pre-existing abnormalities were excluded from the study. Ophthalmology evaluations were conducted again on Day 77. Examinations were conducted by focal illumination and indirect opthalmoscopy and in subdued light after induction of mydriasis.

5. **Neurobehavioral assessment:** A neurobehavioral test battery, consisting of functional observational battery assessments (FOB) and motor activity (MA), was conducted on rats prior to test substance administration to obtain baseline measurements, and again during Weeks 4, 8, and 13.

a. **Functional observational battery:** Testing was counterbalanced by sex and dose over time to minimize the influence of uncontrolled factors. The experimenter conducting the FOB was blind with respect to the treatment group of each animal. Measurements/evaluations of rats were performed in this order: inside the home cage, upon removal from the home cage and while being handled, in a standard open field arena (approximately 85 x 59 x 20 cm), motor activity, and pupil response. The scoring criteria for the FOB were provided on pages 318-321. The duration of the open field evaluation, environmental conditions, and further details concerning the conduct of the FOB were not provided. The following CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
X	Gait/coordination abnormalities	X	Ease of removal	X	Rearing+
X	Convulsions*	X	Lacrimation* / chromodacryorrhea	X	Arousal / general activity level*
X	Tremors*	X	Salivation*	X	Convulsions*
X	Abnormal movements*	X	Piloerection*	X	Tremors*
X	Palpebral closure*	X	Fur appearance	X	Abnormal movements*
	Feces consistency	X	Palpebral closure*	X	Urination / defecation*
	Vocalization		Respiratory rate + and abnormalities		Appearance
	SENSORY OBSERVATIONS	X	Red/crusty deposits*	X	Gait abnormalities / posture*
X	Approach response+	X	Mucous membranes /eye /skin color		Gait score*
X	Touch response+	X	Eye prominence*		Bizarre / stereotypic behavior*
X	Startle response*	X	Muscle tone*		Backing
X	Pain response*	X	Vocalization		Reduced limb function
X	Pupil response*		Convulsions	X	Vocalization
	Corneal reflex		Tremors	X	Muscle spasms/fasciculation
	Palpebral membrane reflex		Hyper/hypothermia	X	Respiration ease
	Pinna reflex		Miosis/mydriasis	X	Respiration rate
X	Righting reflex+		Stains around mouth or nose	X	Palpebral closure
	Olfactory orientation	X	Emaciation		Sides pinched in
	Splay reflex	X	Dehydration		**NEUROMUSCULAR OBSER.**
	Visual placing response		Abdominal tone		Hindlimb extensor strength
			Urination/defecation	X	Forelimb grip strength*
				X	Hindlimb grip strength*
			PHYSIOLOGICAL OBSER.	X	Landing foot splay*
		X	Body weight*		Rotarod performance
		X	Body temperature+		Time to tail flick

*Required parameters; +Recommended parameters

Fore- and hindlimb grip strength were measure by a strain gauge device (Chatillon® -Digital Force gauge), 3 trials per animal per session. Hindlimb splay was assessed by inking the hind paws and releasing the rat from a height of approximately 32 cm onto a piece of paper that covered a padded surface. Heel to heel distance was measured from the inked impressions and recorded. Rectal body temperature was recorded with an YSI Precision™ 4000 Thermometer and temperature probe.

b. Locomotor activity: Immediately after completion of the FOB, the movements of each rat were monitored by an infrared sensor during a 60 minute period, with the number of movements tabulated at 10 minute intervals. An automated activity recording apparatus (Coulbourn® Instruments) monitored time spent moving and number of movements. A continuous movement, regardless of its duration, was counted as one movement. The duration of movement was analogous to "counts" in other types of devices that employ interruption of light beams.

6. Plasma concentrations of DPX-MAT28 and IN-LXT69: On Day 56, plasma samples were collected from 5/sex/dose rats. The blood samples were processed to plasma and analyzed using

high performance liquid chromatography with detection by tandem mass spectrometry (LC/MS/MS) for DPX-MAT28 and the metabolite IN-LXT69.

7. **Hematology and clinical chemistry:** Blood for all measurements but coagulation parameters was collected from the orbital sinus of 10 animals/sex on in the morning of sacrifice (Days 94-95 for males and Days 96-97 for females) from animals fasted after 3 p.m. the previous day. Blood for coagulation parameters was collected at sacrifice from the abdominal *vena cava* and processed to serum. Bone marrow smears were prepared at sacrifice and stained with Wright-Giemsa stain. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	Microscopic blood smear examination
X	(Thromboplastin time)		
X	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
X	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

8. **Urinalysis*:** Urine was collected from animals placed in metabolism cages the day before

sacrifice (Days 94-95 for males and Days 96-97 for females). Animals were fasted and urine collected on the morning of sacrifice. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Optional for 90-day oral rodent studies

9. Sacrifice and pathology

a. Subchronic Toxicity Study: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+	X	**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	X	**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+	X	**OTHER**
	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
X	**RESPIRATORY**	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*	X	Mammary gland*		
X	Nose*	X	Cervix		
X	Pharynx*	X	Vagina		
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

The testes, epididymides, and eyes were fixed in a modified Davidson's solution. All other tissues were fixed in 10% neutral buffered formalin. All tissues collected from the control group (0 ppm) and high exposure group (18,000 ppm) were processed to slides and evaluated microscopically by a veterinary pathologist, as were all tissues from the 6000 ppm female decedent. Tissues were processed, embedded in paraffin, sectioned at 5-6 micron thickness, and

stained with hematoxylin and eosin (H&E). Since examination of tissues did not result in test substance-related findings, the 600, 2000, and 6000 ppm dose groups were not examined.

b. Subchronic Neurotoxicity Study: On Days 94-95, the rats that were designated for neuropathology (5 rats/sex/dose group) were anesthetized by intraperitoneal injection of Nembutal® sodium solution and underwent whole-body *in situ* perfusion in accordance to DuPont Haskell SOP (details not provided). Animals were observed grossly, and the following CHECKED (X) tissues were collected from the perfused animals.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN	X	**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Cerebrum (including hippocampus)		Sciatic notch
X	Midbrain		
X	Cerebellum		
X	Pons		
X	Medulla oblongata		**OTHER**
		X	Sural nerve
		X	Tibial nerve
	SPINAL CORD		Peroneal nerve
X	Cervical swelling	X	Cervical dorsal root ganglion
	Thoracic	X	Cervical dorsal root fibers
X	Lumbar swelling	X	Cervical ventral root fibers
	OTHER	X	Lumbar dorsal root ganglion
X	Gasserian ganglion	X	Lumbar dorsal root fibers
X	Optic nerve	X	Lumbar ventral root fibers
X	Eyes		
X	Gastrocnemius muscle		

The brain, spinal cord, eye (with optic nerve), and gastrocnemius muscle were paraffin embedded, section at approximately 5 µm, and stained with H&E. Central nervous system tissues were also stained with Luxol Fast Blue/Periodic Acid Schiff for myelin sheaths and neuronal bodies. The following samples were embedded in glycol methacrylate: cervical and lumbar dorsal root fibers and ganglia and ventral root fibers; sciatic, tibial and sural nerves; and gasserian ganglia. These samples were then sectioned at approximately 3 µm and stained with H&E. Only tissues from the right side of the animals were processed, and other tissues were saved in appropriate fixative as wet tissue. Tissues from rats in the control and 18,000 ppm groups were processed and examined microscopically. Brain weights were measured, but brain width and length were not measured.

10. Positive controls: Positive control data were not provided with this study. Rather, historical data previously submitted to the Agency were cited that demonstrates the ability of the testing laboratory to detect major neurotoxic endpoints (effects on FOB parameters, motor activity, and peripheral and central nervous system pathology) with positive controls. In MRID 47053501, carbaryl was administered to 10 Crl:CD(SD)IGS(BR) female rats/dose at 0 or 100

mg/kg orally (by gavage) and FOB and motor activity studies were conducted prior to exposure and 30-90 minutes after dosing. No test substance-related effects were observed on foot splay, forelimb strength, or hindlimb grip strength. In the FOB, autonomic signs (lacrimation, labored breathing, polyuria, salivation, absent/low pupilary response) and neuromuscular changes (tremors, muscle fasiculations, muscle spasms, slow righting reflex, altered gait/coordination manifested as uncoordinated movement, splayed rear limbs, low carriage) were observed. Animals were also docile (easy to handle, did not resist removal from home cage, limp muscle tone, low arousal) and exhibited a diminished response to external stimuli (tail pinch, approach and touch, auditory stimulus of finger snap). Quantitative effects on motor activity assessed for 60-90 minutes after dosing with carbaryl showed a significant decrease in the mean duration of movements (↓90-92%) and mean number of movements (↓73-78%). These effects of carbaryl on FOB and motor activity were consistent with previous studies at this facility and in the published literature. Effects on learning and memory were assessed with scopolamine hydrochloride (0, 10 mg/kg i.p.) in the same animals in a water maze 30-60 minutes after dosing (carbaryl could not be used due to motor effects that inhibited swimming). Scopolamine-treated animals made significantly more errors per animal per trial than controls. This effect remained following scopolamine treatment 1 week later, which is consistent with the amnesic effects of scopolamine. The ability to detect effects on foot splay, forelimb and hindlimb grip strength, and adverse pathological effects on the peripheral nervous system were confirmed in the acrylamide administration study (MRID 44660601). Acrylamide was administered to 10 Crl:CD(BR) females rats/dose at 0, 40, or 50 mg/kg (i.p.) on eleven occasions over a 4-week period. Increased foot splay and decreased forelimb strength and hindlimb grip strength were observed in the 50 mg/kg group on Day 24 and increased foot splay was observed in the 40 and 50 mg/kg groups on Days 12 and 24. Peripheral nervous system pathology (axonal and myelin degeneration with phagocytosis and Schwann cell hypertrophy in the sciatic and tibial nerves) was observed at 50 mg/kg. The ability to detect central nervous system pathology was confirmed in a study with trimethyl tin (MRID 44628701). Eight or 10 Crl:CD(BR) male rats were administered 0 or 7.3mg/kg trimethyl tin chloride (6 mg/kg as the free base) i.p. on two consecutive days. Surviving animals were necropsied on Day 6. Microscopic effects of neuronal necrosis and microgliosis were observed in the brain and spinal cord of the animals treated with trimethyl tin. These results are consistent with the published literature. Taken together, these studies adequately demonstrate the ability of the testing laboratory personnel to detect relevant neurotoxic endpoints.

II. RESULTS

A. OBSERVATIONS

1. Mortality: No treatment-related mortality was observed. One control male and one 6000 ppm female were accidentally killed during blood collection just prior to necropsy. All other animals survived until their scheduled sacrifice.

2. Clinical signs of toxicity: No treatment-related effect was observed on clinical signs.

3. Ophthalmology evaluation: No test substance-related ophthalmological signs were noted in male or female rats at any dose.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Decreases ($p \leq 0.05$; unless otherwise noted) in body weights were observed at 18,000 ppm in the males from Days 28-91 (↓6-9%, not statistically significant [NS] at Days 56 and 70) and in the females on Days 56, 70, and 77 (↓8-10%; Table 2). All other mean body weight values in the treated groups were similar to controls. Decreased ($p \leq 0.05$) weekly body weight gains were sporadically observed in the 18,000 ppm group, and overall (Days 0-91) body weight gain was decreased ($p \leq 0.05$) by 15-18%. Decreased ($p \leq 0.05$) body weight gain was observed for one weekly interval each in the 600 ppm females and the 6000 ppm males and females, but these transient effects were considered incidental.

TABLE 2. Mean (±SD) body weights and body weight gains in rats fed aminocyclopyrachlor in the diet for 13 weeks [a]

Day(s)	Dose (ppm)				
	0	600	2000	6000	18,000
Males					
0	256.4±11.3	254.7±13.3	253.7±13.7	255.3±13.0	253.0±10.7
28	429.4±31.9	424.7±24.9	424.0±30.6	416.1±36.2	401.7±17.2* (↓6)
91	580.7±50.4	572.9±43.6	580.3±51.4	578.9±53.0	529.4±34.9* (↓9)
Days (0-91)	324.3±45.0	318.1±36.5	326.7±41.5	323.6±42.7	276.4±30.0* (↓15)
Females					
0	179.4±10.2	176.7±8.8	180.2±9.8	173.7±10.1	174.6±9.8
56	276.8±24.1	273.8±22.4	279.4±27.9	262.7±18.3	254.0±22.4* (↓8)
77	293.7±24.3	292.2±26.1	302.9±31.7	279.9±25.9	264.7±24.1* (↓10)
91	294.6±30.5	288.6±24.6	304.9±34.5	284.1±23.4	270.6±22.6
Days (0-91)	115.2±26.9	111.9±19.3	124.7±26.1	112.5±17.5	94.9±16.9* (↓18)

a Data (n=14-15) were obtained from Tables 1-4 on pages 43-50 in MRID 47573403. Percent difference from controls is included in parentheses, and was calculated by the reviewers.

* Significantly different ($p \leq 0.05$) from the control group

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: No adverse, treatment-related effect was observed on food consumption. Minor decreases ($p \leq 0.05$) in food consumption were observed at 18,000 ppm during Days 0-7, 21-28, and 42-49 in males (↓7-8%) and during Days 70-77 in females (↓11%); and overall (Days 0-91) food consumption was decreased (NS) by only 2-5%.

2. Compound consumption: Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

3. Food efficiency: At 18,000 ppm, decreased ($p \leq 0.05$) food efficiency was noted in males during Days 21-28, 70-77, and 77-84 (↓18-73%) and in females during Days 62-70 (↓112%).

Overall (Days 0-91) food efficiency was decreased ($p \leq 0.05$) by 11% in males and 17% in females. Decreased ($p \leq 0.05$) food efficiency was also noted in the 6000 ppm males on Days 21-28 and the 600 ppm females on Days 84-91, but these transient effects were considered incidental.

D. NEUROBEHAVIORAL RESULTS: No treatment-related effect was observed during the neurological evaluations.

1. Functional observational battery (FOB): A minor decrease ($p \leq 0.05$) in the mean body temperature of the 18,000 ppm females (36.5°C) was noted compared to controls (37.3°C); however, 36.5°C was within the range of control means for the evaluations at baseline and Weeks 4 and 8. Therefore, this difference was considered incidental. No treatment-related effects were noted on fore- or hindlimb grip strength, hindlimb footsplay, rearing, or any of the other behavioral parameters assessed in the FOB for any dose group of either sex.

2. Motor activity (MA): No treatment-related effects were observed in any dose group on the duration of movement or number of movements for any 10-minute interval or for the overall 60-minute session (Table 3). Habituation was observed in all groups for all time points, and was unaffected by treatment.

TABLE 3a. Mean (±SD) duration of movement (seconds) in rats fed aminocyclopyrachlor in the diet for 13 weeks [a]

Week	Dose (ppm)				
	0	600	2000	6000	18,000
Males					
Pre-test	1121+347	960+293	1255+328	914+212	1023+299
4	1546+428	1770+435	1850+354	1518+301	1215+402
8	1541+248	1673+338	1715+267	1298+465	1323+323
13	1368+339	1502+358	1435+269	1257+221	1173+182
Females					
Pre-test	1040+290	829+269	1041+341	1176+286	1137+336
4	1308+364	1246+400	1369+399	1596+571	1436+508
8	1311+339	1340+368	1369+360	1322+419	1411+334
13	1265+328	1182+300	1282+482	1365+354	1187+449

a Data (n=10) were obtained from Tables 19-20 on pages 83-84 in MRID 47573403. No statistical difference was detected.

TABLE 3b. Mean (±SD) number of movements in rats fed aminocyclopyrachlor in the diet for 13 weeks[a]

Week	Dose (ppm)				
	0	600	2000	6000	18,000
Males					
Pre-test	529+150	396+108	525+148	441+86	526+175
4	689+93	676+106	725+134	740+110	627+224
8	702+87	742+142	771+117	622+158	711+161
13	638+75	676+132	662+126	642+113	649+104
Females					
Pre-test	545+138	452+162	505+150	528+111	533+112
4	664+133	650+154	626+168	679+190	630+107
8	698+120	736+168	679+142	657+144	691+92
13	682+117	674+119	647+186	670+96	620+128

a Data (n=10) were obtained from Tables 21-22 on pages 85-86 in MRID 47573403. No statistical difference was detected.

E. PLASMA CONCENTRATIONS OF DPX-MAT28 AND IN-LXT69

DPX-MAT28 was the most abundant metabolite in male and female rats, with the highest concentrations at 10, 268 and 13, 428 ng/mL for 18,000 ppm male and female rats, respectively. In contrast, plasma levels of IN-LXT68 were lower for this dose group at concentrations of 82 and 80 ng/mL for males and females, respectively. The average concentration of DPX-MAT29 in plasma was higher in females than in males at all dose levels. This is consistent with the higher dietary intake of DPX-MAT28 in females than males.

The IN-LXT69 plasma concentrations were below the limit of quantitation for the 600 ppm dietary concentration. The average IN-LXT69 plasma concentrations appeared to be similar for males and females. There was no apparent plateau for the DPX-MAT28 of IN-LXT68 plasma concentrations when compared to dietary intake levels (e.g. plasma concentrations increased with increasing concentration in the diet).

F. SACRIFICE AND PATHOLOGY

1. Organ weight and brain weight: At the terminal sacrifice, there were no test substance-related organ weight effects. Individual and mean organ weight differences were considered spurious or related to body weight.

In males, a small (9%) decrease in the mean final body weight of the 18,000 ppm rats, as compared to the control value, as associated with a similar decrease in the mean absolute liver, heart, spleen, and thymus weights. Some of these decreases were statistically significant. However, there were no gross or microscopic pathology correlates, and most mean relative (% body weight) organ weights were comparable to controls. Therefore, these changes in mean absolute organ weight were interpreted to be a result of the decreased mean body weight and not an indication of an organ-specific effect.

There was a statistically significant decrease (↓12%) in the mean relative (to % brain weight) liver weight in the 18,000 ppm males compared to controls. This difference was also attributed to the decrease in final body weight and secondary to the decrease in absolute liver weights.

No effects on brain weights were observed in either sex.

2. **Gross pathology and neuropathology:** No treatment-related effects were observed during necropsy on gross pathology or neuropathology.

3. **Microscopic pathology and Neuropathology:** No treatment-related microscopic effects in the brain or other organs were observed. In the 18,000 ppm group, the following microscopic findings were noted (# affected/5 treated vs. 0/5 controls): (i) minimal macrophage aggregates in the gastrocnemius muscle in males (1); and (ii) minimal retinal fold/rosette in males (1). No other microscopic finding was reported.

III. DISCUSSION AND CONCLUSIONS

INVESTIGATOR'S CONCLUSIONS: The NOAEL was 6000 ppm, based on decreased body weights, body weight gains, and food efficiency in both sexes at 18,000 ppm.

REVIEWER'S COMMENTS: All animals survived to the day of scheduled sacrifice. One control male and one 6000 ppm female were accidentally killed during blood collection just prior to necropsy.

At 18,000 ppm, decreases ($p \leq 0.05$; except as noted) in body weights were observed in the males from Days 28-91 (↓6-9%, NS at Days 56 and 70) and in the females on Days 56, 70, and 77 (↓8-10%). Decreased ($p \leq 0.05$) weekly body weight gains were sporadically observed in both sexes, and overall (Days 0-91) body weight gain was decreased ($p \leq 0.05$) by 15-18% in both sexes. Significant decreases ($p \leq 0.05$) in food consumption were observed at 18,000 ppm during Days 0-7, 21-28, and 42-49 in males (↓7-8%) and during Days 70-77 in females (↓11%). Decreased ($p \leq 0.05$) food efficiency was noted in males during Days 21-28, 70-77, and 77-84 (↓18-73%) and in females during Days 62-70 (↓112%). Overall (Days 0-91) food efficiency was decreased ($p \leq 0.05$) by 11% in males and 17% in females.

No treatment-related effects were observed on mortality, clinical signs, functional observational battery, motor activity, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis, organ weights, or gross or histological anatomic or neuropathology. There were no effects of the test substance on brain weights or gross or microscopic neuropathology in either sex at any dose.

In males, a small (9%) decrease in the mean final body weight of the 18,000 ppm rats, as compared to the control value, as associated with a similar decrease in the mean absolute liver, heart, spleen, and thymus weights. Some of these decreases were statistically significant. However, there were no gross or microscopic pathology correlates, and most mean relative (% body weight) organ weights were comparable to controls. There were no effects on organ weights that were considered adverse. No effects on organ weights were observed in females. There were no test substance related microscopic effects in either sex.

The plasma concentrations of DPX-MAT28 and the metabolite IN-LXT69 were measured on Day 56 by high performance liquid chromatography with detection by tandem mass spectrometry (LC/MS/MS, MRID 47560007). DPX-MAT28 was the most abundant metabolite in male and female rats, with the highest concentrations at 10, 268 and 13, 428 ng/mL for 18,000 ppm male and female rats, respectively. In contrast, plasma levels of IN-LXT68 were lower for this dose group at concentrations of 82 and 80 ng/mL for males and females, respectively. The average concentration of DPX-MAT29 in plasma was higher in females than in males at all dose levels. This is consistent with the higher dietary intake of DPX-MAT28 in females than males. The IN-LXT69 plasma concentrations were below the limit of quantitation for the 600 ppm dietary concentration. The average IN-LXT69 plasma concentrations appeared to be similar for males and females. There was no apparent plateau for the DPX-MAT28 of IN-LXT68 plasma concentrations when compared to dietary intake levels (e.g. plasma concentrations increased with

increasing concentration in the diet).

The LOAEL is 18,000 ppm (1044.6/1424.9 mg/kg/day), based on decreased body weights, body weight gains, and food efficiency in both sexes. The NOAEL is 6000 ppm (349.4/448.0 mg/kg/day).

The LOAEL for neurotoxicity was not observed. The NOAEL is 18,000 ppm (1044.6/1424.9 mg/kg/day) in both sexes.

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) and a subchronic neurotoxicity study (OPPTS 870.6200b; OECD 424) in rats.

C. **STUDY DEFICIENCIES:** For the 90-day oral toxicity study (870.3100), no deficiencies were observed.

For the subchronic neurotoxicity study (870.6200b), the following deficiencies were considered minor and do not affect the conclusions of the DER or the acceptability of the study:

- The duration of the open field evaluation, environmental conditions, and further details concerning the conduct of the FOB were not provided.
- Gait score and bizarre/stereotypic behavior were not scored during open field observations.

DATA EVALUATION RECORD

DPX-MAT28 (AMINOCYCLOPYRACHLOR)

Study Type: OPPTS 870.3100 [§82-1a], Subchronic Oral Toxicity Study in Mice

Work Assignment No. 5-1-209 C (MRIDs 47560010 and 47560011)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity in Mice [feeding]; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Anand, S.S. (2008) DPX-MAT28 Technical: subchronic toxicity 90-day feeding study in mice. E.I. du Pont de Nemours and Company, Newark, DE . Laboratory Project ID: DuPont-21491, July 2, 2008. MRID 47560010. Unpublished.

Mawn, M.P. (2008) DPX-MAT28 Technical: subchronic toxicity 90-day feeding study in mice (supplement 1). E.I. du Pont de Nemours and Company, Newark, DE. Laboratory Project ID: DuPont-21491, August 21, 2008. MRID 47560011. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRIDs 47560010 and 47560011), Aminocyclopyrachlor (92.2% a.i.; Batch# DPX-MAT28-009) was administered to 15 CD-1 mice/sex/dose group in the diet at dose levels of 0, 300, 1000, 3000, or 7000 ppm (equivalent to 0/0, 47/61, 154/230, 459/649, and 1088/1623 mg/kg/day, in males/females) for approximately 90 days (95/96 days in males/females). At Day 60, blood was collected from the orbital sinus or tail vein of each mouse from the satellite groups (5/sex/concentration) for the determination of plasma concentration of the test substance (DPX-MAT28) and metabolite (IN-LXT69).

There were no treatment-related deaths during the study. Small but statistically significant decreases in body weights were seen for males starting at Day 49 and continuing through the end of treatment at doses of 1000, 3000, and 7000 ppm (↓4-8%). Significant decreases in body weights (↓8-11%) were observed for females at 1000, 3000, and 7000 ppm. Body weight gains

from Day 0-91 were significantly decreased in males (↓12-27%) at 1000 and 7000 ppm. Decreases in body weight gains were not statistically significant for females. There were no effects on food consumption for either sex. Overall food efficiency (Days 0-91) was significantly decreased (↓24%) in the 7000 ppm males and in females at 1000 ppm and 7000 ppm (↓28-36%), but not at 3000 ppm.

The number of reticulocytes in the 7000 ppm females was significantly decreased ($p<0.05$) by 15% compared to controls, but this was not considered adverse.

Absolute and relative (to brain weight) changes in organ weights were observed, but these were not considered adverse due to a lack of corroborating macroscopic or microscopic effects. These changes were: decreased absolute and relative heart weights (↓17-19%) at 7000 ppm compared to controls, increased relative (to body weight) brain weights in the 1000 ppm males, decreased relative (to brain) heart weights in the 1000 ppm females, and increased relative (to body weight) kidney weights in the 1000 ppm females.

The LOAEL was not observed. The NOAEL is 7000 ppm (equivalent to 1088/1623 mg/kg/day in males/females).

The plasma concentration of the parent DPX-MAT28 and the metabolite IN-LXT69 were measured on Day 60. Plasma concentrations of DPX-MAT28 were increased in all treated groups and was slightly higher in males than females at all dose levels. The IN-IXT69 metabolite was not detected in any samples.

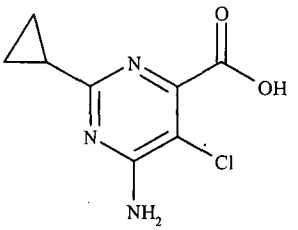
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in mice.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aminocyclopyrachlor

Description:	White solid
Batch #:	DPX-MAT28-009
Purity:	92.2% a.i. (doses adjusted for purity)
Stability:	Stable in the diet for up to 14 days when stored at room temperature or 21 days when stored refrigerated
CAS # of TGAI:	858956-08-8
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Mouse
Strain:	Crl:CD1(ICR)
Age/weight at study initiation:	Approximately 45 days old/25.7-33.0 g males and 19.0-27.0 g females
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Individually in stainless steel, wire mesh cages suspended above cage boards.
Diet:	Certified Rodent LabDiet #5002®, (PMI Nutrition International, LLC, St. Louis, MO), *ad libitum*; except overnight prior to blood collection
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air changes:	Not reported
Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	14 days

B. STUDY DESIGN

1. In-life dates: Start: February 8, 2007 End: May 16, 2007

2. Animal assignment: Mice were selected based on adequate body weight gain and freedom from any clinical signs of disease or injury or ophthalmology abnormalities. They were distributed by computerized, stratified randomization into the study groups presented in Table 1 so that there were no statistically significant differences among group body weight means within a sex. The weight variation of selected mice did not exceed ±20% of the mean weight for each sex. For each dose group, the first 10 mice were designated as main study animals, and the last 5 animals were designated as satellite animals for the evaluation of test substance and metabolite concentration in plasma.

TABLE 1. Study design [a]

Test group	Dietary concentration in M/F (ppm) [c]	Achieved intake in M/F (mg/kg/day)	Main study # Males/Females	Satellite groups [b] # Males/Females
Control	0	0/0	10	5
Low	300	47/61	10	5
Mid-low	1000	154/230	10	5
Mid-high	3000	459/649	10	5
High	7000	1088/1623	10	5

a Data were obtained from page 13 and from Tables 10 and 11 on pages 50 and 52 of MRID 47560010.
b On Day 60, blood was collected from 5 mice/sex/group for evaluation of plasma concentrations of the parent compound and/or its metabolites.
c Dietary concentrations (w/w) were adjusted for the sponsor-supplied purity (92.2% a.i.)

3. **Dose-selection rationale:** It was stated that the test material, DPX-MAT28 technical, is a metabolite of IN-KJM44. In a previous 28-day feeding study conducted with IN-KJM44, mice were dosed with up to 7000 ppm (equivalent to 1149/1429 [M/F] mg/kg/day) in the diet[1] No adverse or treatment-related effects were observed on body weight, nutritional parameters, clinical observations, hematology, clinical chemistry (plasma total protein), gross pathology, or histopathology in either sex. No test substance-related effects were noted for beta-oxidation activity, total cytochrome P450 content, or cytochrome P450 isozyme profile. Dietary concentrations of 0, 300, 1000, 3000, or 7000 ppm were selected for the current study, based on the results of the previous study.

4. **Treatment preparation, administration, and analysis:** The test diets were prepared by grinding the test material, sieving it through a #30 mesh screen, and thoroughly mixing the appropriate amount (adjusted for purity) directly with the basal diet. Control diets were mixed for the same period of time. Test diets were prepared weekly and stored refrigerated until used. Test diets stored beyond 21 days after preparation were not fed to the study animals. From the initial diet preparation, duplicate samples were taken from the top, middle, and bottom of the mixer for each dietary concentration for analysis of concentration and homogeneity of the test material in the diets. Concentration analyses for this preparation were reported as the average of the top, middle, and bottom of these samples. Additionally, the concentration of each dietary level was verified near the middle (Day 48) and end (Day 90) of the study. The stability of the test material in the diet was confirmed at 300 and 18,000 ppm in the concurrently submitted 90-day rat study (MRID 47573403). Stability was determined following room temperature storage for up to 14 days and refrigerated storage for up to 21 days.

[1] DuPont Haskell (2006). IN-KJM44: Repeated Dose Oral Toxicity 28-Day Feeding Study in Mice. Unpublished report, DuPont- 17314.

Results

Homogeneity

300 ppm: Initial analysis = 101.0% nominal and a coefficient of variation (C.V) of 13%. Re-analysis of this sample one month later after frozen storage resulted in a concentration of 89.3% nominal and a C.V. of 7%.

1000 ppm: 98.6% nominal; 8% CV
3000 ppm: 94.0% nominal; 4% CV
7000 ppm: 91.3% nominal; 5% CV

Stability analysis (% of Day 0): 95.9-107% after room temperature storage for 14 days
99.4-117% after refrigerated storage for 21 days

Concentration: 82.9-101.0% nominal

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Body weights, body weight gains, food consumption, food efficiency, clinical pathology, and organ weight data were subjected to statistical analyses. These data were first analyzed using Levene's test for homogeneity of variances and Shapiro-Wilk test for normal distribution. If the results of Levene's test and Shapiro-Wilk test were not significant ($p \geq 0.05$), indicating homogeneous variances and normally distributed data, significant differences among groups were measured using one-way analysis of variance (ANOVA) followed by pair-wise comparisons of the treated groups with controls using Dunnett's test. If the Shapiro-Wilk test was not significant (i.e., data were normally distributed) but Levene's test was significant (i.e., variances were not homogeneous), a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, significant differences among groups were measured using Kruskal-Wallis test followed by pair-wise comparisons of the treated groups with controls using Dunn's test. When an individual observation was recorded as being less than the limit of determination, calculations were performed on half the recorded value. For example, if bilirubin was reported as < 0.1, then 0.05 was used for any calculations performed with that data. When an individual observation was recorded as being greater than the limit of determination, calculations were performed on the recorded value. For example, if specific gravity was reported as >1.083, 1.083 was used for any calculations performed with that data. Significance was denoted at $p \leq 0.05$ or $p \leq 0.01$. The statistical methods were considered appropriate.

C. METHODS

1. Observations

a. **Cage-side observations:** Cage-side observations for mortality, moribundity, and clinical signs of toxicity were performed each morning at approximately the same time each day (±2 hours) and additionally in the afternoon.

b. **Clinical examinations:** At every weighing, each mouse was individually handled and examined for abnormal behavior and appearance. Detailed clinical observations were conducted on each mouse in a standardized arena. These observations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, and presence of clonic, tonic, stereotypical, or bizarre behavior.

2. **Body weight:** All animals were weighed prior to study initiation, on Day 0, weekly throughout the study, and at termination. Body weight gains were reported for each weekly interval throughout the study and for the overall (Days 0-91) study.

3. **Food consumption, food efficiency, and compound intake:** The amount of food consumed by each mouse was measured between weighing intervals, and the mean daily food consumption (g/animal/day) was determined and reported for each weekly interval and for the overall study. Using the food consumption and body weight data, food efficiency (g body weight gain/g food consumption) and test substance intake (mg/kg bw/day) were calculated and were reported for these intervals. Overall test substance intake is reported in Table 1 of this DER.

4. **Ophthalmoscopic examination:** Ophthalmoscopic examinations were conducted on all animals pre-test (Day -13) and prior to termination (Day 92).

5. **Hematology and clinical chemistry:** Blood samples were collected from the abdominal vena cava of each of the main study animals (10/sex/concentration) while under carbon dioxide anesthesia on Days 95-96 for males and Days 96-97 for females. Bone marrow smears were prepared from each animal surviving to scheduled termination; however, these smears were not examined. The following CHECKED (X) parameters were examined in the main study animals:

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Red cell distribution width
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry

	ELECTROLYTES		OTHER
	Calcium		Albumin*
	Chloride		Creatinine*
	Magnesium		Urea nitrogen*
	Phosphorus		Total cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose (fasting)*
	ENZYMES (more than 2 hepatic enzymes)		Total bilirubin
	Alkaline phosphatase (ALK)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin/globulin ratio
	Alanine aminotransferase (ALT/also SGPT)*		
	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Plasma concentrations:** On approximately Day 60, a blood sample (approximately 0.5 mL) was collected from the orbital sinus or tail vein of each mouse from the satellite groups (5/sex/concentration) for the determination of plasma concentration of the test substance (DPX-MAT28) and/or the metabolite (IN-LXT69), as requested by the Sponsor. These plasma samples were analyzed using high performance liquid chromatography with detection by tandem mass spectrometry (LC/MS/MS). The low level calibration standard had an average peak-to-peak response greater than 10x the average noise for control plasma samples. The lower limit of quantitation (LOQ) of the method was calculated by multiplying the low level calibration standard concentration times the minimum sample preparation dilution factor of 20x. The QC fortification recoveries ranged from 91-108%, which demonstrates acceptable analytical method performance.

7. **Urinalysis:** Urinalysis was not conducted. However, these analyses are considered optional for 90-day oral toxicity studies in rodents.

8. **Sacrifice and pathology:** Following blood sampling at study termination, all main study animals were euthanized by exsanguination under carbon dioxide anesthesia and subjected to a gross necropsy. The following CHECKED (X) tissues from all animals were collected. Additionally, the (XX) organs were weighed in all animals surviving until scheduled termination. Paired organs were weighed together.

	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Gall bladder (not rat)*
	Bile duct (rat)
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lungs*
X	Nose*
X	Pharynx*
X	Larynx*

	CARDIOVASC./HEMAT.
X	Aorta*
XX	Heart*+
X	Bone marrow* [a]
X	Lymph nodes*
XX	Spleen*+
XX	Thymus*+
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
X	Prostate*
X	Seminal vesicles*
XX	Ovaries*+
XX	Uterus*+
X	Mammary gland (females)*
X	Vagina

	NEUROLOGIC
XX	Brain*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (and optic nerve)*
	GLANDULAR
XX	Adrenal gland*+
	Lacrimal gland
X	Parathyroid
X	Thyroid*
	OTHER
X	Bone (sternum and/or femur) [b]
X	Skeletal muscle
X	Skin*
X	All gross lesions and masses*
X	Peyer's patches [c]

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
a Bone marrow was collected with the femur and sternum.
b The sternum and femur with knee joint were collected.
c Peyer's patches were collected with the intestines.

Testes, epididymides, and eyes were fixed in modified Davidson's solution. All other tissues were fixed in 10% neutral buffered formalin. All tissues collected from the control and high dose groups were processed routinely, stained with hematoxylin and eosin, and evaluated microscopically. In addition, all tissues from the 1000 ppm male decedent (#307) and all gross lesions were also examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** There were no treatment-related deaths. One male in the 1000 ppm group (#307) was found dead on Day 76; gross and microscopic pathology indicated that the cause of death was due to malignant lymphoma, apparently originating from the thymus with metastasis to the heart, lymph nodes, and thyroid. All other mice survived until scheduled termination.

2. **Clinical signs:** Eye enophthalmus was noted in 2/15 male mice at 7000 ppm from Days 49-91. This finding was minimal in incidence and, along with all of the clinical observations in this study, was stated to be typical for mice of this age and strain. Furthermore, because the ophthalmology examinations revealed no abnormalities in any of the main study animals, the incidences of enophthalmus were not considered treatment-related. All other clinical observations were unrelated to dose.

B. BODY WEIGHTS AND WEIGHT GAINS: Body weights were decreased ($p<0.05$) in both sexes at 1000, 3000, and 7000 ppm compared to controls in males (↓6-8%) and females (↓8-11%). Body weight gains differed significantly ($p<0.05$) from controls in the males at 1000 and 7000 ppm from Day 0-91 (↓25-25). Overall body weight gains of the treated females were comparable to controls.

TABLE 2. Mean (±SD) body weights and body weight gains (g) in mice fed DPX-MAT28 in the diet for up to 91 days. [a]

Parameter/ Time interval		Dose (ppm) 0	300	1000	3000	7000
Males						
Body weight	Day 0	29.5 ± 1.8	29.0 ± 1.7	29.0 ± 1.6	29.2 ± 1.7	29.1 ± 1.4
Body weight	Day 21	33.8 ± 2.1	33.1 ± 2.1	32.1 ± 2.4	31.7 ± 1.9* (↓6)	31.9 ± 1.5
Body weight	Day 49	36.9 ± 2.7	35.7 ± 2.7	34.5 ± 2.5*(↓7)	35.4 ± 1.8	34.7 ± 1.9*(↓6)
Body weight	Day 56	37.8 ± 2.7	36.4 ± 2.8	35.3 ± 2.5* (↓7)	35.7 ± 2.1 (↓6)	35.0 ± 1.9* (↓7)
Body weight	Day 63	38.6 ± 2.7	36.8 ± 3.1	35.6 ± 2.6* (↓8)	36.4 ± 2.3 (↓6)	35.6 ± 1.7* (↓8)
Body weight	Day 77	39.0 ± 2.7	36.9 ± 3.0	35.5 ± 2.5*(↓9)	36.7 ± 2.4	35.7 ± 1.7*(↓8)
Body weight	Day 91	39.5 ± 2.9	37.6 ± 3.3	36.3 ± 2.4* (↓8)	38.1 ± 2.4 (↓4)	36.6 ± 2.0 (↓7)
Body weight gain	Days 7-14	1.6 ± 0.5	1.5 ± 0.6	0.8 ± 0.8*(↓50)	0.5 ± 0.5*(↓70)	0.7 ± 0.5*(↓56)
Body weight gain	Days 14-21	1.2 ± 0.4	1.2 ± 0.5	0.8 ± 0.6	0.4 ± 0.6*(↓67)	0.6 ± 0.7*(↓50)
Body weight gain	Days 49-56	0.9 ± 0.5	0.7 ± 0.6	0.8 ± 0.5	0.4 ± 0.6*(↓56)	0.3 ± 0.5*(↓67)
Body weight gain	Days 70-77	0.1 ± 0.3	0.0 ± 0.4	-0.2 ± 0.5	-0.1 ± 0.5	-0.4 ± 0.4*(↓400)
Body weight gain	Days 0-91	10.4 ± 1.6	8.7 ± 2.6	7.8 ± 2.0* (↓25)	9.2 ± 2.2 (↓12)	7.6 ± 1.6*(↓27)
Females						
Body weight	Day 0	21.8 ± 0.9	22.0 ± 1.1	21.7 ± 1.2	22.2 ± 1.0	22.0 ± 1.2
Body weight	Day 56	27.4 ± 1.9	26.6 ± 1.7	25.2 ± 2.1* (↓8)	26.5 ± 1.5	26.0 ± 2.2
Body weight	Day 63	28.8 ± 1.3	27.4 ± 1.9	25.9 ± 1.8* (↓10)	26.6 ± 1.8	25.9 ± 2.8* (↓10)
Body weight	Day 70	29.3 ± 1.3	27.6 ± 1.7	26.0 ± 1.5* (↓11)	26.9 ± 2.1* (↓8)	26.6 ± 3.0* (↓9)
Body weight	Day 77	29.1 ± 1.5	28.0 ± 2.3	26.3 ± 2.0*(↓10)	27.0 ± 2.4*(↓10)	26.3 ± 3.1*(↓10)
Body weight	Day 84	29.3 ± 1.6	28.0 ± 2.8	26.7 ± 2.2	27.1 ± 2.1	26.4 ± 2.8*(↓10)
Body weight	Day 91	29.3 ± 1.7	28.0 ± 2.3	26.7 ± 1.8 (↓9)	28.5 ± 2.4 (↓3)	27.1 ± 2.9 (↓8)
Body weight gain	Days 0-91	7.1 ± 1.6	6.1 ± 2.1	4.9 ± 1.6 (↓31)	6.4 ± 2.1 (↓10)	5.1 ± 2.1(↓28)

a Data were obtained from Tables 1, 2, 3, and 4 on pages 33-40 of MRID 47560010; n=15 prior to termination of the satellite group on Day 60; thereafter n=10.

* Statistically significant at $p<0.05$.

C. **FOOD CONSUMPTION AND FOOD EFFICIENCY:** Weekly food consumption in the treated males was comparable to controls throughout the study. In the females, the only significant ($p<0.05$) difference in weekly food consumption was an increase of 29% compared to controls at 7000 ppm for Days 84-91. Overall (Days 0-91) food consumption in the treated males and females was comparable to controls. Weekly food efficiency was significantly ($p<0.05$) decreased at 7000 ppm in males compared to controls for several intervals and was decreased (↓24%) from Days 0-91. Overall food efficiency was decreased ($p<0.05$) by 24% in the 7000 ppm males compared to controls.In the females, weekly food efficiency was significantly increased at 7000 ppm for Days 28-35 and at 1000 ppm for Days 84-91 compared to controls. Food efficiency from Days 0-91 was significantly decreased (↓36%) at 1000 ppm and (↓28%) at 7000 ppm. No significant changes were observed at 3000 ppm.

D. **OPHTHALMOSCOPIC EXAMINATION:** No ocular abnormalities were found in any of the surviving main study animals examined on Day 92.

E. **BLOOD ANALYSES**

1. **Hematology:** The number of reticulocytes in the 7000 ppm females was decreased ($p<0.05$) by 15% compared to controls. This finding was considered incidental to treatment because it was minor, not observed in the males, and was not corroborated by any other findings in

hematology or pathology. There were no other dose-related differences from controls in any other hematology parameter in either sex.

2. **Clinical chemistry:** Total protein was comparable to controls in the treated males and females. No other parameters were evaluated.

3. **Plasma concentrations:** Plasma concentrations of DPX-MAT28 were dose-dependently increased in all treated groups, with concentrations of 157, 480, 1376, and 2484 ng/mL in the males and 121, 436, 1249, and 2313 ng/mL in the females fed the 300, 1000, 3000, and 7000 ppm diets respectively (Table 3). Slightly higher plasma concentrations of the parent were noted in the males than in the females at all dose levels. None of the plasma samples detected the metabolite IN-LXT69 concentrations above the limit of quantitation.

TABLE 3. Mean (±SD) plasma concentration (ng/mL) of the parent compound (DPX-MAT28) and IN-LXT69 (a metabolite) in mice fed DPX-MAT28 in the diet for 60 days. [a]

Parameter	Dose (ppm)				
	0	300	1000	3000	7000
Males					
DPX-MAT28	<10.0	157 ± 56	480 ± 106	1376 ± 629	2484 ± 615
IN-LXT69	<10.0	<10.0	<10.0	<10.0	<10.0
Females					
DPX-MAT28	<10.0	121 ± 49	436 ± 188	1249 ± 315	2313 ± 901
IN-LXT69	<10.0	<10.0	<10.0	<10.0	<10.0

a Data were obtained from Table 2 on page 16 of MRID 47560011; n=5.

F. URINALYSIS: Not conducted.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** In the females, absolute and relative (to brain weight) heart weights were decreased by 17-19% at 7000 ppm compared to controls; however, there were no corroborating gross or microscopic findings that would indicate a treatment-related effect. Several other differences in organ weights were noted but were not dose-related and were not corroborated by treatment-related macroscopic or microscopic findings, including: increased relative (to body weight) brain weights in the 1000 ppm males; decreased relative (to brain) heart weights in the 1000 ppm females, and increased relative (to body weight) kidney weights in the 1000 ppm females.

2. **Gross pathology:** No treatment-related findings in gross pathology were found at necropsy. Tail discoloration was noted in one male at 7000 ppm, however, this finding was considered incidental. Incidences of all other gross lesions also were unrelated to dose and only occurred in a single animal per dose group. Enlarged thymus was noted in the 1000 ppm male that died due to malignant lymphoma.

3. **Microscopic pathology:** There were no treatment-related findings in histopathology. All microscopic findings were minimal in incidence and severity and/or were unrelated to dose.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS:

The investigators concluded that there were no adverse effects on in-life, clinical, or pathology parameters in either sex. Therefore, the LOAEL was not observed, and the NOAEL was 7000 ppm (equivalent to 1088 and 1623 mg/kg/day in males and females, respectively).

B. REVIEWER'S COMMENTS:

There were no treatment-related deaths. One male in the 1000 ppm group was found dead on Day 76; gross and microscopic pathology indicated that the cause of death was due to malignant lymphoma, originating from the thymus with metastasis to the heart, lymph nodes, and thyroid. All other mice survived until scheduled termination.

Statistically significant decreases in body weights were seen for males starting at Day 49 and continuing through the end of treatment at doses of 1000, 3000, and 7000 ppm. The magnitude of these body weight decreases were 4-8%. Significant decreases in body weights were observed for females at 1000, 3000, and 7000 ppm. The magnitude of this effect was 8-11%. Body weights from Day 0-91 were decreased in males (↓12-27%) at 1000 ppm, but these decreases were statistically significant only at 1000 ppm and 7,000 ppm. Body weights for females were also decreased from Day 0-91 (↓10-31%) at 1000, 3000, and 7000 ppm, but none of these decreases were statistically significant. There were no decreases in food consumption for either sex. Overall food efficiency was decreased ($p<0.05$) by 24% in the 7000 ppm males compared to controls, reflecting the fact that these animals had a lower body weight gain despite the comparable food consumption. In females, weekly food efficiency was significantly increased at 7000 ppm for Days 28-35 and at 1000 ppm for Days 84-91 compared to controls. Food efficiency from Days 0-91 was significantly decreased (↓36%) at 1000 ppm and (↓28%) at 7000 ppm. No significant changes were observed at 3000 ppm.

In hematological effects, the number of reticulocytes in the 7000 ppm females was decreased ($p<0.05$) by 15% compared to controls. This finding was considered incidental to treatment because it was minor, not observed in the males, and was not corroborated by any other findings in hematology or pathology. Total protein was comparable to controls in the treated males and females.

In the females, absolute and relative (to brain weight) heart weights were decreased by 17-19% at 7000 ppm compared to controls; however, there were no corroborating gross or microscopic finidngs that would indicate an adverse effect. Several other differences in organ weights were noted but were not dose-related and were not corroborated by gross or microscopic findings. These were: increased relative (to body weight) brain weights in the 1000 ppm males, decreased relative (to brain) heart weights in the 1000 ppm females, and increased relative (to body weight) kidney weights in the 1000 ppm females.

In the satellite animals, plasma concentrations of DPX-MAT28 were increased in all treated groups, with concentrations of 157, 480, 1376, and 2484 ng/mL in the males and 121, 436, 1249, and 2313 ng/mL in the females fed the 300, 1000, 3000, and 7000 ppm diets respectively. Slightly higher plasma concentrations of the parent were noted in the males than in the females at all dose levels. None of the plasma samples detected the metabolite IN-LXT69 concentrations above the limit of quantitation.

The LOAEL was not observed. The NOAEL is 7000 ppm (equivalent to 1088/1623 mg/kg/day in males/females).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in mice.

C. STUDY DEFICIENCIES:

The following deficiencies were noted but do not affect the conclusions of this DER or the acceptability of the study:

- No blood clotting measurement was determined.
- Total protein was the only clinical chemistry parameter examined.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.3150; §82-1b, Subchronic Oral Toxicity Study in Dogs

Work Assignment No. 5-1-209 D (MRIDs 47560012 and 47560013)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
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EPA Reviewer: Marquea D. King, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley **Signature:** Stephen C Dapson for
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity in Dogs (feeding); OPPTS 870.3150 [§82-1b]; OECD 409.

PC CODE: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Luckett, E.M. (2008) DPX-MAT28 Technical: subchronic toxicity 90-day feeding study in dogs, revised final report. MPI Research, Inc., Mattawan, MI. Laboratory Project ID: DuPont-21489, March 13, 2008, revised July 2, 2008. MRID 47560012. Unpublished.

Mawn, M.P. (2008) DPX-MAT28 Technical: subchronic toxicity 90-day feeding study in dogs, supplement 1. E.I. du Pont de Nemours and Company, Newark, DE. Laboratory Project ID: DuPont-21489, March 12, 2008, supplement 1, August 13, 2008. MRID 47560013. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In this subchronic oral toxicity study (MRID 47560012), 4 beagle dogs/sex/group were administered aminocyclopyrachlor (DPX-MAT28; 92.2% a.i.; Batch No. DPX-MAT28-009) daily via the diet (400 g offered daily for approximately 2 hours) at doses of 0, 250, 1250, 5000, or 15,000 ppm (equivalent to 0/0, 6.5/7.0, 33/38, 126/124, and 426/388 mg/kg/day in males/females) for 13 weeks.

No treatment related effect was observed on mortality, clinical signs, neurological evaluations, body weight, body weight gains, food consumption, food efficiency, hematology, clinical chemistry, urinalysis, organ weights, or gross or microscopic pathology.

A dose-related increase in the incidence of skin scaling on the feet was observed in males, with 4/4 males affected at 15,000 ppm. Half of the females were affected at the high dose. In most cases, all 4 feet exhibited scaling. The time of onset of scaling in most cases was 41-56 days,

continuing to study termination (Day 91). The clinical severity of this effect was not noted and the foot pads were not biopsied. This effect was not considered adverse, because no impairment in walking or weight bearing and no grooming (e.g. licking/biting) of the feet was observed.

The plasma concentrations of parent increased with dietary intake ranging from 1968-63,000 ng/mL in males and 3193-82,575 ng/mL in females at doses of 250-15,000 ppm. Plasma concentrations were always higher in the females than in the males. The metabolite IN-LXT69 was below the limit of quantitation except at 15,000 ppm, where only minor amounts (11.6-11.7 ng/mL) were noted (MRID 47560013).

Total cytochrome P450 measured was unrelated to dose in both sexes and was 0.320-0.409 nmol/mg in males and 0.378-0.421 nmol/mg in females. At 5000 and 15,000 ppm, induction of the isozymes 1A1 in males (↑402-420%) and 2B1/2 in males (↑91-125%) and females (↑34-93%) was observed ($p \leq 0.05$ at 5000 ppm only). A decreased level of 2E1 was noted in the 15,000 ppm males (↓37%). Although the effect on 2E1 seemed dose-dependent, the noted decrease was not statistically significant and a similar effect was not noted in females. It is unclear if there is an inhibitory effect on 2E1.

The LOAEL was not observed. The NOAEL is 15,000 ppm (equivalent to 426/388 mg/kg/day in males/females).

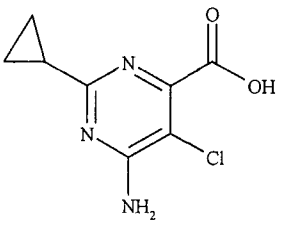
This study is classified **Unacceptable/Guideline** and does not satisfy the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs. An adverse effect was not noted and the limit dose was not tested. This study is **upgradable** pending submission of a satisfactory dose-selection rationale.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28

Description:	Light-white to off-white powder
Batch No.:	DPX-MAT28-009
Purity (w/w):	92.2% a.i.
Stability of compound:	Stable in the diet for up to 14 days when stored at room temperature or 21 days when stored refrigerated
CAS #:	858956-08-8
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Dog
Strain:	Beagle
Age at study initiation:	Approximately 8-9.5 months of age
Mean weight at study initiation:	9.8-10.8 kg males 8.6-9.3 g females
Source:	Covance Research Products, Inc. (Kalamazoo, MI)
Housing:	Dogs were housed individually in suspended, stainless steel cages.
Diet:	Powdered Certified Canine Diet® 5007 (PMI Nutrition International, Inc., St. Louis, MO), 400 g offered daily for approximately 2 hours except when fasted prior to blood collections
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	64-84°F
Humidity:	30-70%
Air changes:	Not provided
Photoperiod:	12 hours light/12 hours dark
Acclimation period:	15 Days

B. STUDY DESIGN

1. In life dates: Start: February 28, 2007; End: May 30, 2007

2. Animal assignment/dose levels: Study groups are presented in Table 1. The females were distributed using a standard, by weight, block randomization procedure, while the males were distributed using a randomization procedure, stratified by total testicular volume. The weight variation of selected dogs did not exceed ±20% of the mean weight for each sex.

TABLE 1: Study design [a]			
Test group	Dietary concentration (ppm)	Compound intake (mg/kg/day in M/F)	No. dogs/sex sacrificed at Week 13 [b]
Control	0	0/0	4
Low	250	6.46/7.02	4
Mid	1250	33.31/37.94	4
Mid-High	5000	126.23/124.12	4
High	15,000	425.71/387.53	4

a Data were obtained from pages 20 and 122-123 of MRID 47560012.

3. **Dose-selection rationale:** It was stated that the Sponsor chose the dose levels based on the results of previous studies conducted with a related chemical. Further information was not provided.

4. **Treatment preparation and administration:** For each dose level, an appropriate amount of the test substance (adjusted for purity) was mixed directly with basal diet to yield the desired concentration. Control diets were mixed for a similar length of time. Test diets were prepared weekly and stored refrigerated until used. Homogeneity (top, middle, bottom) was verified in all dose levels of the first preparation. Stability was determined in the initial batch of the 250 and 15,000 ppm dietary formulations following room temperature or refrigerator storage for up to 14 days. Samples for concentration verification were taken from all dose levels on Weeks 1, 5, 9, and 13. In each case, duplicate samples of the dietary preparation were taken, and samples were frozen until analysis.

Results

Homogeneity (% CV): 4-6%

Stability (% of nominal): 91.3-104.4% after room temperature storage for 14 days
97.3-108.8% after refrigerated storage for 14 days

Concentration (% of nominal): 84.4-119.2%

Dose (ppm)	Range (% of nominal)
250	88.8-103.6
1250	85.6-119.2
5000	84.4-96.0
15,000	84.7-91.3

The analytical data indicate that the mixing procedure was adequate and that the variation between the target and actual dosage to the study animals was acceptable.

5. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure		
	Preliminary tests and transformations	Preliminary test is not significant	Preliminary test is significant
Body weight Body weight gain Food consumption Hematology Coagulation Clinical chemistry Organ weight Leukocyte count Differential leukocyte count	When n>2, Levene's test for homogeneity was performed. Log transformation was applied to the leukocyte and differential leukocyte counts only.	One-way analysis of variance followed by Dunnett's test was done. If n<3, Welch's t-test with Bonferroni correction was performed. When there were only 2 groups involved, the Student's t-test was conducted.	One-way analysis of variance, followed by Welch's t-test with Bonferroni correction, was performed.
Food efficiency Urine volume pH Osmolality	Data were rank-transformed.	A non-parametric equivalent to Dunnett's test was performed when there were more than 2 groups to compare, and the Student's t-test was conducted when only 2 groups were compared.	

Statistical significance was denoted at $p \leq 0.05$ and $p \leq 0.01$, and 2-tailed tests were performed. The statistical methods were considered appropriate as long as the assumptions for parametric testing were confirmed prior to parametric testing.

C. METHODS

1. Observations

a. **Cageside observations:** Animals were inspected at least twice daily for morbidity and mortality.

b. **Clinical examinations:** Detailed clinical examinations were performed daily during acclimation and twice daily (pre-dose and approximately 4 hours after food removal) during the study.

c. **Neurological evaluations:** Neurobehavioral observations (a modified functional observational battery) were conducted prior to randomization and once weekly during the treatment period (approximately 4 hours after food removal). These observations used explicitly defined scales where possible. Further details were not provided. Parameters that were evaluated included posture, clonic and tonic movements, palpebral closure, lacrimation, arousal, stereotypy, bizarre behavior, limb position, gait, mobility, salivation, piloerection, body and limb tone, fore- and hindlimb strength, and pupil response.

2. **Body weight:** All dogs were weighed prior to treatment, weekly throughout the study, and prior to termination. Body weight gains were reported for each interval between body weight measurements and also for Weeks 1 to 13.

3. **Food consumption, food efficiency, and compound intake:** Food consumption (g/animal/day) and food efficiency (%) were reported weekly from Weeks 1-13 and for Weeks 1-13 overall. Compound intake (mg/kg bw/day) values were calculated from the nominal dietary test material concentration, food consumption, and body weight data.

4. **Ophthalmoscopic examination:** Ocular examinations were performed on all dogs prior to treatment and prior to the scheduled termination.

5. **Hematology, clinical chemistry, and urinalysis:** Clinical pathology evaluations were conducted on all animals during Weeks -2, -1, 4, 8, and 12. The animals had access to drinking water, but were fasted overnight prior to sample collection. Blood samples were collected from the jugular vein. The order of bleeding was by alternating one animal from each dose group. Urine samples were collected for at least 16 hours using steel pans placed under the cages. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Red cell distribution width
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total cholesterol*
X	Potassium*	X	Globulin
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg.,*)	X	Total bilirubin* [a]
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (also SGPT)*		
X	Aspartate aminotransferase (also SGOT)*		
X	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

a With direct bilirubin if total bilirubin exceeded 1 mg/dL
* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

c. Urinalysis

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

6. **Determination of aminocyclopyrachlor and the metabolite, IN-LXT69:** Blood was collected from the jugular vein of all animals (non-fasted) during Week 9. Plasma was prepared and frozen. Samples were analyzed for the concentration of aminocyclopyrachlor and IN-LXT69 using LC/MS/MS.

7. **Sacrifice and pathology:** All animals were sacrificed by intravenous overdose of sodium pentobarbital solution, followed by exsanguination and were subjected to a detailed necropsy. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed; bilateral organs were weighed together.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, abdominal*	XX	Brain* +
X	Salivary glands*	XX	Heart* +	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen* +	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus* +		GLANDULAR
X	Ileum*			XX	Adrenal gland* +
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys* +	XX	Parathyroid* [a] +
X	Rectum*	X	Urinary bladder*	XX	Thyroid* [a] +
XX	Liver* +	XX	Testes* +		OTHER
X	Gall bladder* +	XX	Epididymides* +	X	Bone
X	Pancreas*	X	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries* +	X	Skin*
X	Trachea*	XX	Uterus* [a] +	X	Joint, tibiofemoral
X	Lungs*	X	Mammary gland* (females)	X	Nictitans gland
X	Nose*	X	Vagina	X	All gross lesions and masses*
	Pharynx*	XX	Cervix [a]		
X	Larynx*				

a The uterus and cervix were weighed together, as were the parathyroid and thyroid.
* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150
\+ Organ weight required for non-rodent studies.

Testes and eyes (with optic nerves) were fixed in Davidson's solution, while all other tissue samples were fixed in neutral buffered formalin. Formalin was infused into the lung via the trachea. All tissues collected from the control and 15,000 ppm groups and all gross lesions were processed routinely, stained with hematoxylin and eosin (H&E), and examined microscopically. A 4-step grading system was utilized to define gradable lesions for comparison between dose groups. A second pathologist conducted a peer review, consisting of an examination of all tissues determined to be target organs by the study pathologist, all neoplasms, all tissues from 75% of the animals in the 15,000 ppm group, and all tissues from at least 25% of the control group.

8. **Cytochrome P450 assays:** A sample of liver was excised from each animal, snap frozen in liquid nitrogen and stored frozen at -70°C. The samples were homogenized, and microsomes were prepared by differential centrifugation. Total hepatic cytochrome P450 content was measured by spectral analysis according to the method of Omura and Sato. Quantities of the cytochrome P450 isozymes IA1, 1A2, 2B1/2, 2E1, 3A2, and 4A1/2/3 were examined by Western Blot analysis using commercially available isozymes.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All animals survived until scheduled termination.

2. **Clinical signs of toxicity:** A treatment-related sign, scaling of the skin on the feet, was observed (Table 2). The incidence of skin scaling on the feet increased in the 5000 and 15,000 ppm males, both in number of times observed (410-1184 in treated groups vs 0 in controls) and in number of animals (2-4/4 treated vs 0/4 controls). The incidence of an interdigital cyst was increased in the 15,000 ppm females, both in number of times observed (81 in treated groups vs 9 in controls) and in the number of animals (2/4 treated vs 1/4 controls). Information on the severity of these skin conditions was not provided. No other skin-related conditions were observed in either sex. These effects were not associated with any other clinical or pathological response. The incidences of all other clinical findings were unrelated to dose.

TABLE 2. Selected clinical findings (# of times observed/total # of animals affected) in dogs fed aminocyclopyrachlor in the diet for 13 weeks [a]

Finding	Dose (ppm)				
	0	250	1250	5000	15,000
Males					
Skin scaling	0/0	182/1	32/2	410/2	1184/4
Interdigital cyst	0/0	7/1	0/0	6/2	1/1
Females					
Skin scaling	0/0	43/1	464/2	129/2	444/2
Interdigital cyst	9/1	0/0	6/1	0/0	81/2

a Data (n=4) were obtained from Table 1 on pages 44-51 in MRID 47560012.

3. **Neurological evaluations:** No treatment-related effect was observed during the neurological evaluations.

B. BODY WEIGHT AND WEIGHT GAIN:

No adverse, treatment-related effects were observed on body weight or body weight gain (Table 3). Decreased body weight gains were noted during Weeks 1-2 in all treated male groups, but this effect was transient and unrelated to dose.

TABLE 3. Mean (±SD) body weights and body weight gains in dogs fed aminocyclopyrachlor in the diet for 13 weeks [a]

Week(s)	Dose (ppm)				
	0	250	1250	5000	15,000
Males					
1	9.81±1.48	10.77±1.27	10.35±1.25	10.77±1.07	10.19±1.44
7	11.12±1.13	12.49±1.50	11.60±1.64	11.96±1.60	11.42±1.28
13	11.62±0.97	12.92±1.59	12.08±1.71	12.56±1.55	11.91±1.42
Weeks (-1 to 13)	1.80±0.37	2.20±0.99	1.82±0.84	1.97±0.45	1.90±0.62
Females					
1	9.26±0.58	8.56±0.82	8.86±0.86	8.77±0.73	9.06±0.89
7	9.67±0.82	9.60±1.03	10.08±0.96	9.55±0.75	9.76±0.94
13	10.10±0.78	10.39±0.61	10.54±1.00	9.96±0.56	9.92±1.24
Weeks (-1 to 13)	1.26±0.65	1.86±0.57	1.69±0.73	1.34±0.07	1.13±0.57

a Data (n=4) were obtained from Tables 3-5 on pages 84-97 in MRID 47560012. No significant difference ($p \leq 0.05$) was noted between the treated and control groups

C. **FOOD CONSUMPTION AND EFFICIENCY:** No adverse, treatment-related effect was observed on food consumption or efficiency. Transient differences ($p \leq 0.05$) in weekly food consumption relative to controls occurred in both sexes, as did transient decreases in weekly food efficiency in males. These findings were unrelated to dose or occurred prior to treatment. Food consumption and food efficiency over the entire treatment interval (Weeks 1-13) were similar between treated groups and controls.

D. **OPHTHALMOSCOPIC EXAMINATION:** No treatment-related effect was observed during ophthalmoscopic examination.

E **BLOOD ANALYSES**

1. **Hematology:** No treatment-related findings were observed in hematology parameters. Differences ($p \leq 0.05$) were unrelated to dose or occurred prior to treatment.

2. **Clinical chemistry:** No treatment-related findings were observed in clinical chemistry parameters. The only difference ($p \leq 0.05$) between mean values in the treated groups and controls was decreased sorbitol dehydrogenase in the 15,000 ppm females prior to treatment.

F. **DETERMINATION OF AMINOCYCLOPYRACHLOR AND THE METABOLITE, IN-LXT69:** The plasma concentrations of parent increased with dietary intake ranging from 1968-63,000 ng/mL in males and 3193-82,575 ng/mL in females at doses of 250-15,000 ppm (Table 4). Plasma concentrations were always higher in the females than in the males. The metabolite was below the limit of quantitation except at 15,000 ppm, where only minor amounts (11.6-11.7 ng/mL) were noted.

TABLE 4. Mean (±SD) plasma concentration (ng/mL) of the parent compound (DPX-MAT28) and IN-LXT69 (a metabolite) in dogs fed DPX-MAT28 in the diet for 13 weeks. [a]

Parameter	Dose (ppm)				
	0	250	1250	5000	15,000
Males					
DPX-MAT28	<10.0	1968±272	10,398±2091	31,575±6108	63,000±3366
IN-LXT69	<10.0	<10.0	<10.0	<10.0	11.7±1.4
Females					
DPX-MAT28	<10.0	3193±493	14,875±2767	37,650±8060	82,575±9557
IN-LXT69	<10.0	<10.0	<10.0	<10.0	11.6±1.2

a Data were obtained from Table 2 on page 16 of MRID 47560013; n=5.

G. **URINALYSIS:** No treatment-related findings were observed during urinalysis. Differences ($p \leq 0.05$) were observed between the pH of the treated groups (both sexes) and controls; however, this response was unrelated to dose and only occurred during Weeks 12 (males) or 8 (females). Therefore, these responses were considered incidental.

H. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No adverse, treatment-related findings were observed on organ weights. At 1250 ppm, decreased ($p \leq 0.05$) absolute brain weight in males and increased ($p \leq 0.05$) absolute heart weight of females were observed, but were unrelated to dose. Other differences in mean organ weights were noticed between the 15,000 ppm group and controls, such as decreased absolute testicular weights, decreased absolute thymus weights in males, decreased relative to body ovary weights, increased absolute thyroid/parathyroid gland weight in females, and decreased relative to body uterus with cervix weight. However, adverse effects in these organs were not corroborated by gross or histological pathology, and a statistically significant difference was not detected.

2. **Gross pathology:** No treatment-related gross findings were observed.

3. **Microscopic pathology:** No treatment-related microscopic pathology findings were noted.

4. **Cytochrome P450 assays:** Total cytochrome P450 measured was unrelated to dose in both sexes and was 0.320-0.409 nmol/mg in males and 0.378-0.421 nmol/mg in females (Table 5). At 5000 and 15,000 ppm, induction of the isozymes 1A1 in males (↑402-420%) and 2B1/2 in males (↑91-125%) and females (↑34-93%) was observed ($p \leq 0.05$ at 5000 ppm only). A decreased level of 2E1 was noted in the 15,000 ppm males (↓37%). Although the effect on 2E1 seemed dose-dependent, the noted decrease was not statistically significant and a similar effect was not noted in females. It is unclear if there is an inhibitory effect on 2E1. Decreased ($p \leq 0.05$) 4A1/2/3 was noted in the 15,000 ppm males (↓11%) and increased ($p \leq 0.05$) 4A1/2/3 was noted in the 1250 and 15,000 ppm females (↑13-15%). These differences on 4A1/2/3 levels were considered minor.

TABLE 5. Mean (±SD) relative levels of hepatic cytochrome P450 isozymes (as net intensity) in dogs fed DPX-MAT28 in the diet for 13 weeks. [a]

Cytochrome	Dose (ppm)				
	0	250	1250	5000	15,000
Males					
Total cytochrome P450 (nmol/mg)	0.407±0.103	0.409±0.115	0.367±0.047	0.320±0.046	0.405±0.163
1A1	173±78 [b]	255±212	NQ	869±279* [b] (↑402)	899±502 (↑420)
1A2	NQ	NQ	NQ	NQ	NQ
2B1/2	6466±195	8097±303	6673±195	14,537±687* (↑125)	12,333±300 (↑91)
2E1	774±526 [c]	890±300	709±215 [b]	1121 [d]	487±5 [c] (↓37)
3A2	14,061±7742	8054±4453	10,164±4440	8635±4759	10,505±6058
4A1/2/3	110,960±5614	109,584±8517	100,190±2079	102,521±6806	99,175±4382* (↓11)
Females					
Total cytochrome P450 (nmol/mg)	0.421±0.60	0.396±0.078	0.378±0.042	0.394±0.049	0.415±0.054
1A1	781±204	835±407	1086±327	979±270 [b]	1033±176
1A2	NQ	NQ	NQ	NQ	NQ
2B1/2	13,086±651	7477±314	6963±610	25,237±583* (↑93)	17,513±865 (↑34)
2E1	481±120	693±92	900±194* (↑87)	594±300	523±211
3A2	19,631±9223	19,028±12,474	20,628±5434	23,226±3427	22,411±2447
4A1/2/3	104,017±6971	111,144±1494	117,163±5892* (↑13)	113,233±5705	120,065±1655* (↑15)

a Data were obtained from Tables 1 and 2 on pages 804-805 of MRID 47560012; n=4, except as noted. Percent difference from controls is included in parentheses, and was calculated by the reviewers.

b n=3

c n=2

d n=1

NQ Not quantified (no bands or bands too faint)

* Significantly different ($p \leq 0.05$) from the control group

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The NOAEL was 15,000 ppm, the highest dose tested. An adverse effect was not observed.

B. REVIEWER COMMENTS: No treatment related effect was observed on mortality, clinical signs, neurological evaluations, body weight, body weight gains, food consumption, food efficiency, hematology, clinical chemistry, urinalysis, organ weights, or gross or microscopic pathology.

The effect of an increased incidence of skin scaling on the feet was considered dose-dependent in males. This effect was absent in male and female controls, but a dose-related increase was observed in males, will all males affected at the high dose. Half of the females were affected at the high dose. In most cases, all 4 feet exhibited scaling. The time of onset of scaling in most cases was 41-56 days, continuing to study termination (Day 91). The clinical severity of this effect was not noted and the foot pads were not biopsied. This is effect is unusual, since it is usually inherited in canines. However, this effect was not considered adverse because there is no clinical evidence that skin scaling caused impaired function (e.g. difficulty walking), or grooming behaviors (e.g. gnawing at feet, licking feet), or lesions associated with grooming behaviors (e.g. cracks/bleeding from licking or biting).

Interdigital cysts, absent in controls, were also observed in males and females, although this effect was not dose-related

The plasma concentrations of parent increased with dietary intake ranging from 1968-63,000 ng/mL in males and 3193-82,575 ng/mL in females at doses of 250-15,000 ppm. Plasma concentrations were always higher in the females than in the males. The metabolite was below the limit of quantitation except at 15,000 ppm, where only minor amounts (11.6-11.7 ng/mL) were noted.

Total cytochrome P450 measured was unrelated to dose in both sexes and was 0.320-0.409 nmol/mg in males and 0.378-0.421 nmol/mg in females. At 5000 and 15,000 ppm, induction of the isozymes 1A1 in males (↑402-420%) and 2B1/2 in males (↑91-125%) and females (↑34-93%) was observed ($p \leq 0.05$ at 5000 ppm only). A decreased level of 2E1 was noted in the 15,000 ppm males (↓37%). Although the effect on 2E1 seemed dose-dependent, the noted decrease was not statistically significant and a similar effect was not noted in females. It is unclear if there is an inhibitory effect on 2E1.

The LOAEL was not observed. The NOAEL is 15,000 ppm (equivalent to 426/388 mg/kg/day in males/females).

This study is classified **Unacceptable/Guideline** and does not satisfy the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs because a LOAEL was not observed at a non-limit dose (1000 mg/kg/day). This study is **upgradable** pending submission of a satisfactory dose-selection rationale.

C. **STUDY DEFICIENCIES:** An adverse effect was not noted and the limit dose was not tested. Without justification for the dose-selection, this deficiency is considered major. In addition, as a minor deficiency, the pharynx was not examined microscopically.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.3200 [§82-2]; 28-Day Dermal Toxicity in Rats

Work Assignment No. 5-1-209 E (MRID 47560014)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Secondary Reviewer: Marquea D. King, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity in Rats; OPPTS 870.3200 [§82-2]; OECD 410.

PC CODES: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (90.9% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Merkel, D.J. (2008) DPX-MAT28 technical: Dermal toxicity study (28 day repeat dermal application study in rats). Eurofins Product Safety Laboratories, Dayton, NJ. Laboratory Project ID: DuPont-22796, March 28, 2008. MRID 47560014. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a 28-day dermal toxicity study (MRID 47560014), DPX-MAT28 (Aminocyclopyrachlor; 90.9% a.i., Batch # DPX-MAT28-010) was applied to the shaved intact skin of 10 Sprague-Dawley rats/sex/dose at dose levels (adjusted for purity) of 0, 150, 400, or 1000 mg/kg/day (limit dose) for 6 hours/day for 28 consecutive days.

There were no unscheduled deaths. There were no effects of the test substance on ophthalmology parameters. The only clinical sign observed was limited and very slight (barely perceptible) erythema/edema (score of 1 on a scale of 4, 4 being severe) in three males. Animals affected were one male at 400 mg/kg/day on Day 28, one male at 1000 mg/kg/day on Day 3, and one male at 1000 mg/kg/day on Day 28. There were no adverse effects of the test substance on body weight, body weight gain, food consumption, food efficiency, clinical chemistry, hematology, gross pathology, or histopathology.

There were statistically significant, dose-related changes in adrenal gland and ovary weights in 1000 mg/kg/day females and a decrease in kidney weights in females at 150 mg/kg/day that were not considered adverse due to a lack of corroborating microscopic lesions.

The LOAEL was not observed. The NOAEL is 1000 mg/kg/day (limit dose).

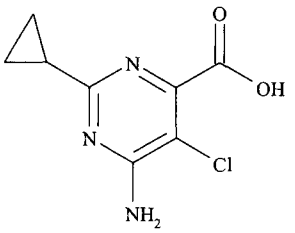
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200; OECD 410) in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Aminocyclopyrachlor (DPX-MAT28)

Description:	White solid
Batch #:	DPX-MAT28-010
Purity:	90.9% a.i.
Stability:	Not reported
CAS #:	858956-08-8
Structure:	

2. **Vehicle:** The test material was applied neat, but moistened with distilled water.

3. **Test animals**

Species:	Rat	
Strain:	Hsd:Sprague-Dawley®	
Age/ weight at study initiation:	Approximately 8-9 weeks/ 243-267 g males and 175-199 g females	
Source:	Harlan (Indianapolis, IN)	
Housing:	Individually in suspended stainless steel cages	
Diet:	Purina Certified Rodent Meal #5002 (St. Louis, MO), *ad libitum*, except for overnight prior to blood collection.	
Water:	Filtered tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	57-67%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	8 days	

B. STUDY DESIGN

1. **In-life dates:** Start: June 13, 2007 End: July 12, 2007

2. **Animal assignment:** Animals were randomly assigned to the test groups noted in Table 1. Body weights were within ±20% of the group mean for each sex.

TABLE 1. Study design [a]			
Group	Dose (mg/kg/day)	# Males	# Females
1	0	10	10
2	150	10	10
3	400	10	10
4	1000	10	10

a Data were extracted from page 13 of the study report.

3. **Dose selection rationale:** It was stated that the Sponsor selected the doses in Table 1, and that 1000 mg/kg/day (limit dose) was expected to potentially exhibit some evidence of toxicity and the NOAEL was expected to be the low or intermediate dose.

4. **Preparation and treatment of animal skin:** Approximately 24 hours prior to initiation of dosing, the fur from the dorsal and lateral areas of the trunk (approximately equal to 10% of total body surface area) was clipped. Hair removal was repeated as necessary thereafter. The applied quantities of the test substance were adjusted weekly based on individual animal body weight; doses were adjusted for compound purity. For Groups 2 and 3, the test substance was applied neat (moistened with distilled water) to 6 in^2 (2 in x 3 in) 4-ply gauze pads, and the gauze pads were then applied to the intact skin and secured to the body with non-irritating tape (3M Micropore™). For Group 4, the moistened test material was applied directly to the 2 in x 3 in test area, covered with a gauze pad, and secured with tape. The dressings were removed after 6 hours, and the application areas were cleaned with water and patted dry. The animals were exposed in this manner for 28 consecutive days.

   Rats in the control group were exposed to distilled water only using the same procedure as described for the treated rats.

5. **Statistics:** The hematology and clinical chemistry data were first analyzed using Levene's test for homogeneity and the Shapiro-Wilk test for normality. If variances were not significantly different, groups were compared using one-way ANOVA followed by Dunnett's t-test. If the Shapiro-Wilk test was not significant, but the Levene's test was significant, a robust version of Dunnett's test was used. If Levene's test was significant, groups were compared using the Kruskal-Wallis test followed by Dunn's test. For all other data, homogeneity of variance and normality were first checked using Bartlett's test. If variances were not significantly different, groups were compared to controls using one-way ANOVA followed by Dunnett's t-test. If Bartlett's test was significant, groups were compared using the Kruskal-Wallis test followed by Dunn's test. The sexes were evaluated separately and significance was denoted at $p \leq 0.05$. The analyses were considered appropriate.

C. METHODS

1. Observations

a. **Cageside observations:** All animals were observed twice daily for mortality and moribundity, and once daily for signs of toxicity. The animals were examined for signs of local skin irritation daily after patch removal using the Draize method.

b. **Detailed examinations:** Detailed examinations (including handling and open-field observations) were conducted once prior to study initiation and weekly thereafter before the daily application of the test material.

c. **Neurological evaluations:** Neurological evaluations were limited to the handling and open-field observations performed weekly. However, a subchronic neurotoxicity study (MRID 47573403) was reviewed concurrently.

2. **Body weight:** Animals were weighed just prior to the first patch application (Days 1 and 2 in males and females, respectively), weekly thereafter, and at termination.

3. **Food consumption and efficiency:** Individual food consumption was determined weekly (adjusting for spillage). Mean daily food consumption (g/animal/day) and food efficiency (%) were calculated.

4. **Ophthalmoscopic examination:** The eyes of all animals were examined prior to initiation of treatment and on Day 24 of the study.

5. **Hematology and clinical chemistry:** Following the 28th application, blood samples for hematology and clinical chemistry were collected via the orbital sinus from all animals (fasted overnight) under isoflurane anesthesia. Additionally on Day 29/30 (males/females), blood samples for evaluation of coagulation parameters were collected via the inferior vena cava under isoflurane anesthesia. The following CHECKED (X) parameters were examined.

a. **Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		Erythrocyte morphology
X	(Activated partial thromboplastin time)	X	Red cell distribution width
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Inorganic phosphorus	X	Total cholesterol*
X	Potassium* (K)	X	Globulins
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes*)	X	Total bilirubin
X	Alkaline phosphatase (AP)*	X	Total protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT)*		
X	Aspartate aminotransferase (AST)*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
X	Sorbitol dehydrogenase*		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

6. **Urinalysis:** Urinalysis was not performed and is not required by the guideline.

7. **Sacrifice and pathology:** All animals were sacrificed by exsanguination under isoflurane anesthesia and were subjected to a gross pathological examination. The following CHECKED (X) tissues were collected for possible histological examination. The (XX) organs, additionally, were weighed. Paired organs were weighed together.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+		Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and femur)
	Bile duct* (rat)	X	Prostate*		Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin* (treated & untreated areas)
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*	X	Mammary gland*		
X	Nose*		Oviducts		
X	Pharynx*	X	Vagina		
X	Larynx*				

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
\+ Organ weights required

The eyes, epididymides, and testes were preserved in modified Davidson's solution, and all other tissues were preserved in neutral buffered formalin. The collected tissues from the control and 1000 mg/kg/day animals were routinely processed, embedded in paraffin, sectioned (4 μm), and stained with hematoxylin and eosin. Slide preparation and histopathological assessment were performed by Histo-Scientific Research Laboratories.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All animals survived to scheduled sacrifice.

2. **Clinical signs of toxicity:** No treatment-related clinical signs were observed at any dose in either sex.

3. **Dermal irritation:** Incidence of dermal irritation was limited to very slight erythema at the dose site in 3 males (one each at 400 mg/kg/day on Day 28 and at 1000 mg/kg/day on Days 3 and 28). No signs of dermal irritation were observed in the females during the study.

B. BODY WEIGHT AND WEIGHT GAIN: Statistically significant differences from control were limited to an increase (↑5%, $p \leq 0.05$) in body weight noted in the 1000 mg/kg/day females on Day 29 (Table 2). Likewise, body weight gain (calculated by reviewers) was increased by 31% in this group for the overall (Days 2-29) study period. These findings were not considered adverse.

TABLE 2. Mean (±SD) body weights and body weight gain (g) in rats dermally exposed to DPX-MAT28 for 4 weeks. [a]

Day	Dose (mg/kg/day)			
	0	150	400	1000
Males				
1	255.8±7.4	257.4±6.4	255.7±6.1	255.8±6.5
28	336.2±14.1	331.2±21.1	324.2±11.8	339.6±19.0
Overall gain [b]	80.4	73.8	68.5	83.8
Females				
2	184.7±6.3	183.1±6.2	182.1±4.9	183.6±6.7
29	222.8±7.4	220.3±8.9	222.9±8.7	233.6±8.3* (↑5)
Overall gain [b]	38.1	37.2	40.8	50 (↑31)

a Data were extracted from Table 2 on page 23 of the study report; n=10. Percent difference from controls (calculated by the reviewers) is presented parenthetically.

b Calculated by reviewers from data within this table.

C. FOOD CONSUMPTION and EFFICIENCY: No adverse treatment-related effects on food consumption or food efficiency were observed in either sex. The statistically significant decreases in food consumption noted in the 150 and 400 mg/kg/day females were minor and not dose-dependent.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related ocular lesions were observed during the study.

E. BLOOD ANALYSES

1. **Hematology:** No treatment-related effects were observed in any hematology parameter. The increased ($p \leq 0.05$) red cell distribution width (↑6%) noted in the 150 mg/kg/day females was considered minor.

2. **Clinical chemistry:** No adverse treatment-related effects were observed in any clinical chemistry parameter. The decreases ($p \leq 0.05$) in creatinine (↓13-16%) noted in the 150 and 1000 mg/kg/day females were minor and not dose-dependent.

F. SACRIFICE AND PATHOLOGY

1. **Organ weight:** In the 1000 mg/kg/day females, absolute adrenal weight was increased ($p<0.05$) by 15% compared to controls. As there were no corroborative gross or histopathological findings this finding was not considered to be related to treatment. In the 150 mg/kg/day females, decreases ($p\leq0.05$) were noted in absolute ovarian weights (↓20%), relative (to body) ovarian weights (↓19%), and relative (to brain) ovarian weights (↓ 22%). Relative (to brain) kidney weight were also decreased (↓10%) in females at 150 mg/kg/day, However, these findings were not dose-related and lacked any corroborative gross or histopathological findings. All absolute and relative organ weights in the males were similar to controls.

2. **Gross pathology:** No treatment-related gross lesions were observed at any dose in either sex.

3. **Microscopic pathology:** No treatment-related microscopic lesions were observed at any dose in either sex.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** The investigators concluded that dermal exposure to DPX-MAT28 at up to 1000 mg/kg/day (limit dose) for 6 hours/day for 28 consecutive days did not induce any adverse effects in rats. The NOAEL was 1000 mg/kg/day.

B. **REVIEWER COMMENTS:** There were no unscheduled deaths. There were no effects of the test substance on ophthalmology parameters. The only clinical sign observed was limited and very slight (barely perceptible) erythema/edema (score of 1 on a scale of 4, 4 being severe) in three males. Animals affected were one male (#7042) at 400 mg/kg/day on Day 28, one male (#7065) at 1000 mg/kg/day on Day 3, and one male (#7069) at 1000 mg/kg/day on Day 28. There were no adverse effects of the test substance on body weight, body weight gain, food consumption, food efficiency, clinical chemistry, hematology, or gross or histopathology.

There was a significant ($p<0.05$) increase (↑15%) in adrenal gland weights in females at 1000 mg/kg/day. Also, there were significant decreases in the absolute, relative (to body), and relative (to brain) ovarian weights (↓19-22%) and relative (to brain) kidney weights ((↓10%) of 150 mg/kg/day females. These findings at 150 mg/kg/day were not dose-related. Since there were no corroborating microscopic lesions, these effects in the adrenal gland, ovaries, and kidneys were not considered adverse.

The LOAEL was not observed. The NOAEL is 1000 mg/kg/day (limit dose).

C. STUDY DEFICIENCIES: The following deficiencies were noted that did not affect the validity of the study.

- For Groups 2 and 3, the test substance was applied to the gauze pads, not directly to the skin. This could result in reduced contact of the test substance with the skin. The test substance was directly applied to the skin in Group 4 (the high dose group).
- There was no assessment of motor activity, grip strength, or sensory reactivity to visual, auditory, or propiceptive stimuli. However, a subchronic neurotoxicity study (MRID 47573403) that included these parameters was reviewed concurrently.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.3700a [§83-3a]; Developmental Toxicity Study in Rats

Work Assignment No. 5-01-209 F (MRID 47560016)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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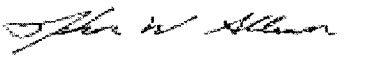
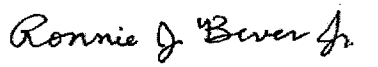
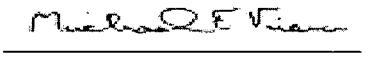
Primary Reviewer: John W. Allran, M.S.	Signature:  Date: 03/07/09
Secondary Reviewer Ronnie J. Bever Jr., Ph.D.	Signature:  Date: 03/07/09
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: 03/07/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature:  Date: 03/07/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Secondary Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Work Assignment Manager: Myron Ottley **Signature:**
Risk Assessment Branch III, Health Effects Division (7509P) **Date:** 10/15/2009

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study in Rats (gavage); OPPTS 870.3700a [§ 83-3a]; OECD 414.

PC CODE: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Lewis, J.M. (2008). DPX-MAT28 Technical: Developmental toxicity study in rats. E.I. du Pont de Nemours and Company, Newark, DE and Critical Path Services, LLC, Wilmington, DE. Laboratory Project ID: DuPont-22378, February 21, 2008. MRID 47560016. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 47560016), Aminocyclopyrachlor (92.2%; Batch #DPX-MAT28-9-009) in 0.5% methylcellulose was administered via daily oral gavage in a dose volume of 5 mL/kg to 25 time-mated presumed pregnant Sprague-Dawley rats/dose group at doses of 0, 30, 100, 300, or 1000 mg/kg/day from gestation days (GD) 6-20. On GD 21, all maternal rats were euthanized; each dam's uterus was removed via cesarean section and its contents examined. The fetuses were examined for external, visceral, and skeletal malformations and variations.

All maternal animals survived until scheduled termination. No clinical signs of toxicity were noted in any of the dams. Body weights, body weight gains, and food consumption in the treated groups were comparable to controls throughout the study. The only gross abnormality was liver discoloration in a single dam at 100 mg/kg/day, and this finding was considered incidental because it was minimal in incidence and not observed at the high dose.

The maternal LOAEL was not observed. The maternal NOAEL is 1000 mg/kg/day (limit dose).

There were no abortions, premature deliveries, late resorptions, complete litter resorptions, or dead fetuses. There were no effects of treatment on the numbers of litters, live fetuses, or early resorptions. Furthermore, sex ratio and post-implantation losses in the treated groups were comparable to controls.

There was no evidence of altered growth or development. Fetal body weights in the treated groups were comparable to controls.

There were no treatment-related external, visceral, or skeletal variations or malformations. Skeletal variations in the skull, vertebrae, ribs, and sternebrae were unrelated to dose. One skeletal malformation (fused cervical vertebrae) was found at 300 mg/kg/day. The only other malformations were: anophthalmia and eye bulge in one 300 mg/kg/day fetus and protruding tongue in another 300 mg/kg/day fetus. These findings were considered incidental because they only occurred in individual fetuses and were not observed at the high dose.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day (limit dose).

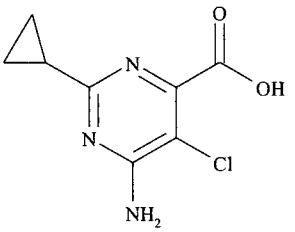
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.3700a; OECD 414) for a developmental toxicity study in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Aminocyclopyrachlor

Description:	White solid
Batch #:	DPX-MAT28-9-009
Purity:	92.2% a.i.
Compound stability:	Stable in the vehicle for up to 5 hours at room temperature
CAS #:	858956-08-8
Structure:	

2. **Vehicle:** 0.5% methylcellulose

3. **Test animals**

Species:	Rat
Strain:	Crl:CD(SD)
Age/ weight range at GD 0:	Approximately 67 days; 222-270 g females
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Individually in stainless steel wire mesh cages suspended above cage boards
Diet:	Certified Rodent LabDiet® 5002 (PMI® Nutrition International, LLC, St. Louis, MO), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** Not reported **Photoperiod:** 12 hours light : 12 hours dark
Acclimation period:	Approximately 4 days

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: September 9, 2007 End: October 31, 2007

2. **Mating:** Nulliparous time-mated females were received from the supplier on gestation day (GD) 0, 1, 2, 3, or 4. It was stated that the day that mating was confirmed was designated GD 0; however, the method for determining positive evidence of mating (e.g., presence of a vaginal plug and/or sperm in a vaginal smear) was not described.

3. **Animal assignment:** Presumed pregnant dams were assigned the treatment groups shown in Table 1 using a randomization procedure designed to produce a homogeneous distribution of body weights across groups within each breeding lot.

TABLE 1. Animal assignment [a]					
Dose (mg/kg/day)	0	30	100	300	1000
No. females	25	25	25	25	25

a Data were obtained from page 12 of MRID 47560016.

4. **Dose-selection rationale:** The doses used in the current study were selected based on the results of a previously conducted range-finding developmental toxicity study in rats with a similar compound (methyl ester of DPX-MAT28).[1]. In this study, 8 dams/dose group were administered doses of 0, 25, 300, or 1000 mg/kg/day via daily oral gavage from gestation days (GD) 6-20 and body weight, food consumption, and clinical signs observed. On GD 21, all maternal rats were euthanized; each dam's uterus was removed via cesarean section, gravid uterine weight was recorded, and uterine contents examined. The fetuses were examined externally, but visceral and skeletal examinations were not performed. It was stated that there were no clinical signs or treatment-related effects on any maternal or developmental endpoints at any dose level.

5. **Dose preparation, administration, and analysis:** The test formulations were prepared daily by suspending the appropriate amount of the test substance, adjusted for purity, in 0.5% methylcellulose. The dosing suspensions were stored at room temperature until use. The dose suspensions were administered daily via oral gavage from GD 6-20 in a dose volume of 5 mL/kg body weight, based on the most recent body weight. Near the beginning of the study, four independent samples were collected from each concentration. Three of these samples were collected from the top, middle, and bottom of the dose preparation; these samples were used to verify concentration and determine homogeneity. The fourth sample was held for 5 hours at room temperature and analyzed to determine the stability of the test material in the dose formulations. Additionally near the end of the study, concentration analyses were performed on the one sample from each dose level.

Results

Homogeneity (% CV): 3-9%

Stability (% nominal): 112-114% (calculated by reviewer)

Concentration (mean % nominal): 82.1-103%

The analytical data indicate that the test substance was homogeneous and stable in the suspensions and that the variation between the nominal and actual dosage to the animals was acceptable.

C. OBSERVATIONS

1 DuPont Haskell. (October, 2004). H-26568: Pilot Developmental Toxicity Study in Rats. Unpublished Data. DuPont-15563. Work Request Number (WR) 15372, Study Service Code (SC) 840.

1. **Maternal observations and evaluations:** Cage-side checks for mortality and moribundity were conducted at least once daily during the quarantine and pretest periods and twice daily (AM and PM) during treatment. Clinical observations were conducted on GD 4 and twice daily during GD 6-20 (during weighing and approximately 2-3 hours post-dosing). Each animal was weighed on GD 4 and daily from GD 6-21. Mean maternal body weight changes and food consumption were reported for GD 6-8, 8-10, 10-12, 12-14, 14-16, 16-18, 18-20, 20-21, and for the overall (GD 6-21) treatment period. Additionally, it was stated that food consumption was recorded on GD 4; however, these data were not presented. Food spillage of >5 g was recorded. On GD 21, all dams were euthanized by carbon dioxide asphyxiation and exsanguination. A gross necropsy was performed on each dam, and the liver was removed and weighed. Gross lesions were retained for possible histopathologic examination in the event that test substance-related effects were observed. (However, since no test substance-related effects were observed, microscopic examination was not conducted). The uterus and ovaries were removed via cesarean section. The numbers of corpora lutea in each ovary were counted. The uterine contents were examined, and the numbers and relative positions of implantations, early resorptions, late resorptions, live fetuses, and dead fetuses were recorded. Uteri without visible implantations were immersed in 10% aqueous ammonium sulfide to detect very early resorptions. Implantations were classified as follows:

Live fetus:	Fully formed and responds to stimuli
Dead fetus:	Fully formed with little or no evidence of maceration
Late resorption:	Identifiable structures (i.e., digital rays)
Early resorption:	No visible fetal structures

2. **Fetal evaluations:** Each live fetus was weighed, sexed, and examined for external abnormalities. Approximately half of the live fetuses in each litter were euthanized using an intraperitoneal injection of a commercial euthanasia agent (not specified) and examined for visceral alterations by fresh tissue dissection[2]. The remaining fetuses were euthanized by decapitation, and the frozen heads were examined using a serial sectioning technique[3]. Additionally, it was stated that all live fetuses with external malformations were subjected to visceral examination, and decapitation of these fetuses for head examination was conducted at the discretion of the study director or designee.

D. DATA ANALYSIS

1. **Statistical analyses:** The following statistical analyses were performed. Significance was denoted at $p<0.05$. The statistical methods were considered appropriate. For litter parameters, the proportion of affected fetuses per litter or the litter mean was used the experimental unit for statistical evaluation.

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2 Staples, R.E. (1974). Detection of visceral alterations in mammalian fetuses. *Teratology* **9(3)**, A37-A38.

3 Astroff A.B. et. al. (2002). Frozen-sectioning yields similar results as traditional methods for fetal cephalic examination in the rat. *Teratology* **66(2)**, 77-84.

Parameter	Method of Statistical Analysis		
	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Maternal body weight Maternal body weight change Maternal food consumption Corpora lutea Implantations Live fetuses Dead fetuses Resorptions	Levene's test for homogeneity of variance and Shapiro-Wilk test for normality [a]	One-way analysis of variance and Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Incidence of pregnancy Maternal mortality Females with total resorptions Early deliveries	None	Sequential application of Cochran-Armitage test [b]	
Incidence of fetal alterations	None	Exact Mann-Whitney with a Bonferroni-Holm adjustment	
Fetal weight (Covariates: litter size, sex ratio) Sex ratio (Covariate: litter size)	Levene's test for homogeneity and Shapiro-Wilk test for normality [c]	Analysis of covariance and Dunnett-Hsu	Non-parametric analysis of covariance

a If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was followed by Dunn's test.
b If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact test with a Bonferroni correction was used.
c A normalizing, variance stabilizing transformation was used as needed.

2. **Indices:** The following indices were reported in Appendix K on pages 154-159 of the study report:

   Pre-implantation loss (%) = (# corpora lutea – # implantations)/ # corpora lutea x 100

   Post-implantation loss (%) = (# implantations – live fetuses)/ # implantations x 100

   Live fetuses as % of implantations (%) = # live fetuses/ # implantations x 100

3. **Historical control data:** No historical control data were provided. The registrant was contacted about providing historical control data. They did not think it was necessary because there were no effects.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical signs:** All maternal animals survived until scheduled termination. No treatment-related clinical signs were noted in any of the dams. Hair loss was reported in all dose groups except for the 1000 mg/kg/day animals. Brown-stained fur was found on the chin of one rat at 1000 mg/kg/day on GD 19. Wet fur on the chin/perinasal region was

observed in one rat in the 100 mg/kg/day group on GD 18. No other clinical signs were noted.

2. **Body weights and body weight gains:** Maternal body weights and body weight gains of the treated groups were comparable to controls throughout the study (Table 2).

TABLE 2. Mean (±SD) maternal body weights and body weight gains (g) [a]

Parameter/Interval	Dose in mg/kg bw/day (# of Dams)				
	0 (25)	30 (25)	100 (25)	300 (25)	1000 (25)
Body weights					
GD 6	245.3 ± 12.4	245.3 ± 10.3	247.3 ± 11.4	245.3 ± 10.0	245.0 ± 10.0
GD 21	392.6 ± 19.9	394.6 ± 21.8	397.8 ± 23.3	393.5 ± 24.2	386.6 ± 25.7
Corrected body weight GD 21 [b]	303.2 ± 15.8	303.9 ± 16.6	308.9 ± 20.7	306.0 ± 17.3	297.7 ± 16.6
Body weight gains					
Overall BW gain: GD 6-21	147.3 ± 12.9	149.3 ± 17.9	150.6 ± 17.8	148.3 ± 18.6	141.6 ± 22.6
Gravid uterine weight (g)	89.4 ± 9.6	90.7 ± 15.1	88.9 ± 12.4	87.5 ± 17.7	88.9 ± 15.1
Corrected BW gain: GD 6-21 [c]	57.9 ± 10.2	58.6 ± 13.7	61.7 ± 15.4	60.7 ± 13.9	52.7 ± 14.4

a Data were obtained from Tables 2 and 3 on pages 26-28 and Appendix G on pages 125-130 of the study report.
b Corrected (net) body weight = terminal body weight – gravid uterine weight
c Corrected body weight gain = net body weight on GD 21 – body weight on GD 6

3. **Food consumption:** Maternal food consumption in the treated groups was comparable to controls throughout the study.

4. **Gross pathology:** Aside from a single dam at 100 mg/kg/day with liver discoloration, no gross abnormalities were found in any animal at any dose level. This finding was considered incidental to treatment.

5. **Cesarean section data:** Summary data from the cesarean sections are presented in Table 3. There were no abortions, premature deliveries, late resorptions, complete litter resorptions, or dead fetuses. There were no effects of treatment on the numbers of litters, live fetuses, or early resorptions. Furthermore, fetal body weights, sex ratio, and post-implantation losses in the treated groups were comparable to controls.

TABLE 3. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)				
	0	30	100	300	1000
# Animals assigned (mated)	25	25	25	25	25
# Animals pregnant	25	25	25	25	25
Pregnancy rate (%)	100	100	100	100	100
# Nonpregnant	0	0	0	0	0
Maternal wastage					
No. died	0	0	0	0	0
No. died pregnant	0	0	0	0	0
No. died nonpregnant	0	0	0	0	0
No. aborted	0	0	0	0	0
No. premature delivery	0	0	0	0	0
Total no. corpora lutea	337	319	339	343	343
Corpora lutea/dam	13.5 ± 3.1	13.3 ± 2.6 [b]	13.6 ± 3.0	13.7 ± 2.0	13.7 ± 2.2
Total no. implantations	292	306	298	307	307
(Implantations/dam)	11.7 ± 1.3	12.2 ± 2.2	11.9 ± 1.9	12.3 ± 1.7	12.3 ± 2.1
Total no. litters	25	25	25	25	25
Total no. live fetuses	290	304	293	293	297
(Live fetuses/dam)	11.6 ± 1.3	12.2 ± 2.2	11.7 ± 2.0	11.7 ± 2.7	11.9 ± 2.1
Total no. dead fetuses	0	0	0	0	0
(Dead fetuses/dam)	0	0	0	0	0
Total no. resorptions	2	2	5	14	10
Early	2	2	5	14	10
Late	0	0	0	0	0
Resorptions/dam	0.08 ± 0.28	0.08 ± 0.28	0.20 ± 0.50	0.56 ± 2.02	0.40 ± 0.65
Early	0.08 ± 0.28	0.08 ± 0.28	0.20 ± 0.50	0.56 ± 2.02	0.40 ± 0.65
Late	0	0	0	0	0
Complete litter resorptions	0	0	0	0	0
Mean fetal weight (g), males	5.89 ± 0.28	5.70 ± 0.30	5.77 ± 0.26	5.67 ± 0.41	5.66 ± 0.27
females	5.55 ± 0.26	5.38 ± 0.31	5.52 ± 0.28	5.28 ± 0.48	5.44 ± 0.32
Sex ratio (% male) [c]	47 ± 14	47 ± 15	47 ± 17	54 ± 16	51 ± 15
Pre-implantation loss (%) [d]	10.8 ± 12.9	7.8 ± 12.1	10.4 ± 11.6	9.6 ± 12.1	10.0 ± 13.4
Post-implantation loss (%) [d]	0.6 ± 2.3	0.6 ± 1.9	1.7 ± 4.4	4.6 ± 16.8	3.2 ± 5.3
Live fetuses as % implantations (%)	99.3	99.3	98.3	95.4	96.7

a Data were obtained from Table 7 on pages 32 and 33 and Appendix K on pages 154-159 of the study report.
b n = 25 in all groups for all parameters, with the exception of the number of corpora lutea in the 30 mg/kg/day group (n=24).
c The reviewers multiplied the provided sex ratio (males/females) by 100 to present the data as a percentage.
d For pre- and post-implantation losses, the group mean standard deviation (with associated mean) was calculated by the reviewers from individual data presented in Appendix K on pages 154-159 of the study report.

B. DEVELOPMENTAL TOXICITY

1. **External examinations:** All external findings are presented in Table 4. At external examination, one fetus in the 300 mg/kg/day group had a small eye bulge of the right eye, which upon closer examination corresponded with anophthalmia in this animal. Another fetus in this group had a protruding tongue, a malformation. These findings were considered incidental because they only occurred in individual fetuses and were not observed at the high

dose. There were no other external findings.

TABLE 4. External findings [# fetuses (litters) affected] [a]

Observation	Dose (mg/kg bw/day)				
	0	30	100	300	1000
No. fetuses (litters) examined - body	290 (25)	304 (25)	293 (25)	293 (25)	297 (25)
No. fetuses (litters) examined - head	140 (25)	145 (25)	140 (25)	142 (25)	143 (25)
Malformations					
Tongue, protruding	---	---	---	1 (1)	---
Anophthalmia	---	---	---	1 (1) [b]	---
Gross finding					
Eye bulge, small	---	---	---	1 (1) [b]	---

a Data were obtained from Table 8 on pages 34 and 35 and Appendix M on page 320 of the study report.
b Findings denoted by this superscript were observed in the same fetus (L04 from Dam #419).
--- No animals affected (i.e., zero incidence)

2. **Visceral examinations:** All visceral findings are presented in Table 5. One fetus at 30 mg/kg/day had a discolored liver, a variation. Discolored intestines were noted as a gross finding in one fetus in the 100 mg/kg/day group. These findings were considered incidental because they only occurred in individual fetuses and were not observed at the high dose. There were no other visceral findings.

TABLE 5. Visceral findings [# fetuses (litters) affected] [a]

Observation	Dose (mg/kg bw/day)				
	0	30	100	300	1000
No. fetuses (litters) examined	140 (25)	145 (25)	140 (25)	142 (25)	143 (25)
Variation					
Liver, discolored	---	1 (1)	---	---	---
Gross finding					
Intestines, discolored	---	---	1 (1)	---	---

a Data were obtained from Table 8 on page 36 of the study report.
--- No animals affected (i.e., zero incidence)

3. **Skeletal examination:** Incidences of all skeletal malformations and variations are presented in Table 6. There were no treatment-related skeletal findings. The only skeletal malformation was a fused cervical arch in the vertebra(e) of one fetus in the 300 mg/kg/day group. Because this finding only occurred in a single animal and was not observed at the high dose, it was considered incidental. Incidences of incomplete ossification of the bones of the skull were observed, in addition to several variations in the vertebrae (unossified and/or bipartite ossification of the thoracic centrum), ribs (short, cervical, full supernumerary, short supernumerary, thickened, wavy, and/or extra ossification site), and sternebrae (unossified, misaligned, and/or fused). However, the incidences of all of these variations were unrelated to dose.

TABLE 6. Skeletal findings [# fetuses (litters) affected] [a]

Observation	Dose (mg/kg bw/day)				
	0	30	100	300	1000
No. fetuses (litters) examined - body	290 (25)	304 (25)	293 (25)	293 (25)	297 (25)
No. fetuses (litters) examined - head	150 (25)	159 (25)	153 (25)	151 (25)	154 (25)
Malformations					
Vertebrae – cervical arch, fused	---	---	---	1 (1)	---
Variations					
Skull – incomplete ossification					
Frontal	---	---	---	5 (1)	---
Zygomatic	---	---	1 (1)	---	---
Interparietal	---	---	---	1 (1)	---
Supraoccipital	3 (2)	2 (2)	1 (1)	3 (2)	---
Parietal	---	1 (1)	1 (1)	2 (2)	---
Vertebrae					
Thoracic centrum					
unossified	---	---	---	1 (1)	---
bipartite ossification	6 (6)	1 (1)	2 (2)	1 (1)	8 (6)
Ribs					
Short	2 (2)	1 (1)	5 (2)	1 (1)	2 (1)
Cervical	3 (2)	3 (1)	4 (2)	1 (1)	---
Full supernumerary	3 (1)	---	---	---	---
Short supernumerary	7 (2)	2 (2)	2 (2)	---	2 (1)
Thickened	3 (1)	---	---	---	---
Wavy	4 (1)	---	1 (1)	---	---
Extra ossification site	19 (10)	13 (6)	10 (9)	9 (4)	5 (5)
Sternebrae					
Unossified	---	---	---	4 (2)	---
Misaligned	---	---	2 (2)	---	1 (1)
Fused	1 (1)	---	---	1 (1)	---

a Data were obtained from Table 8 on pages 37-39 of the study report.
--- No animals affected (i.e., zero incidence)

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATOR'S CONCLUSIONS:** It was concluded that the NOAEL for maternal and developmental toxicity was 1000 mg/kg/day, the highest dose level tested. There were no treatment-related deaths, clinical signs of toxicity, or gross pathology findings. The mean number of corpora lutea, implantation sites, resorptions, live fetuses, sex ratio, and fetal weights were comparable across all groups. There were no test substance-related fetal external, visceral, or skeletal malformations or variations observed at any dose level.

B. **REVIEWERS' COMMENTS**

1. **Maternal toxicity:** All maternal animals survived until scheduled termination. No clinical signs of toxicity were noted in any of the dams. Body weights, body weight gains, and food consumption in the treated groups were comparable to controls throughout the study. The only gross abnormality was liver discoloration in a single dam at 100 mg/kg/day, and this finding was considered incidental because it was minimal in incidence and not observed at the high dose.

The maternal LOAEL was not observed. The maternal NOAEL is 1000 mg/kg/day (limit dose).

2. **Developmental toxicity**

a. **Deaths/resorptions:** There were no abortions, premature deliveries, late resorptions, complete litter resorptions, or dead fetuses. There were no effects of treatment on the numbers of litters, live fetuses, or early resorptions. Furthermore, sex ratio and post-implantation losses in the treated groups were comparable to controls.

b. **Altered growth:** There was no evidence of altered growth or development. Fetal body weights in the treated groups were comparable to controls. The observed incidences of incomplete ossification of the skull and unossified thoracic centrum and sternebrae were not observed at the high dose.

c. **Developmental variations:** There were no treatment-related external, visceral, or skeletal variations. Several variations in the vertebrae, ribs, and sternebrae were found but were unrelated to dose.

d. **Malformations:** There were no treatment-related external, visceral or skeletal malformations. The only malformations noted were: anophthalmia and fused cervical vertebrae in one 300 mg/kg/day fetus and protruding tongue in another 300 mg/kg/day fetus. These findings were considered incidental because they only occurred in individual fetuses and were not observed at the high dose.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day (limit dose).

This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3700a; OECD 414) for a developmental toxicity study in rats.

C. **STUDY DEFICIENCIES:** The following deficiency was noted but does not affect the acceptability of this study or the conclusions of this DER.

Historical control data were not provided. The registrant was contacted about providing historical control data. They did not think it was necessary because there were no effects.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.3700b [§83-3b]; Developmental Toxicity Study in Rabbits

Work Assignment No. 5-01-209 G (MRID 47560015)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
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EPA Secondary Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10-14-2009
EPA Work Assignment Manager: Myron Ottley **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study in Rabbits (gavage); OPPTS 870.3700b [§83-3b]; OECD 414.

PC CODE: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Fleeman, T.L. (2008) A prenatal developmental toxicity study of DPX-MAT28 in rabbits. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-189193, February 14, 2008, revised July 14, 2008. MRID 47560015. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, DuPont Haskell[SM] Laboratory for Health and Environmental Sciences, Stine-Haskell Research Center, P.O. Box 50, Newark, DE

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 47560015), Aminocyclopyrachlor (92.2% a.i.; Lot #MAT28-009) in 0.5% methylcellulose was administered daily via oral gavage in a dose volume of 4 mL/kg to 22 time-mated presumed pregnant New Zealand White rabbits/dose group at doses of 0, 100, 300, 500, or 1000 mg/kg/day from gestation days (GD) 7-28. On GD 29, all maternal rabbits were euthanized; each dam's uterus was removed via cesarean section and its contents examined. The fetuses were examined for external, visceral, and skeletal malformations and variations.

There was one maternal death at 1000 mg/kg/day on GD 13 that was considered treatment-related. Increased respiration was observed 1 hour after dosing on GD 7 in this animal, and rales and decreased defecation were noted during GD 9-13. This female had a body weight loss of 394 g from GD 7-13 and consumed ≤14 g food/day for 4-5 days prior to death. Also at 1000 mg/kg/day, two animals aborted (one on GD 20 and the other on GD 26). Clinical findings noted for both females included decreased defecation several days prior to abortion and red material in the cage pan or anogenital area on the day of abortion. These abortions may be secondary to

body weight loss >10%. These females exhibited body weight losses of 636-643 g (17-19%) from GD 7 through the day of abortion. These maternal effects corresponded with reduced food consumption, generally ≤17 g/day from GD 13 or 17 through the day of abortion.

There was one death at 1000 mg/kg/day unrelated to treatment that was attributed to a gavage error and another death at 100 mg/kg/day of unknown cause. One 300 mg/kg/day animal aborted on GD 28. Clinical findings noted for this female prior to abortion included 11 occurrences of decreased defecation and 3 occurrences of soft stool. This female consumed ≤18 g of food per day between GD 18-19 and 27-28, but had no notable effects on body weight gain. This abortion at 300 mg/kg/day was not considered an effect of treatment because it was not dose-related (no abortions at 500 mg/kg/day). Aside from the above-mentioned clinical signs associated with mortality and abortion, no treatment-related clinical signs were observed. The only treatment-related gross findings at necropsy were in animals that died or aborted. These findings included red skin matting around the urogenital or nasal areas, dark red contents in the vagina, and white precipitate in the abdominal soft tissue.

Reductions in food consumption were statistically significant only in the 1000 mg/kg/day group. These reductions were consistently about 30% after GD 14-15 through the end of the study. Decreases in body weight gain were statistically significant only at GD 14-17, although the decreases throughout the remainder of the study are probably of biological significance. This consistent relationship between decreased food consumption and decreased body weight gain were not observed in the 500 mg/kg/day group. In this group, no significant differences in body weight gain were observed and there were no significant decreases in food consumption.

The maternal LOAEL is 1000 mg/kg/day (limit dose) based on mortality, decreased body weight gains and food consumption, and abortion. The maternal NOAEL is 500 mg/kg/day.

At 1000 mg/kg/day, there were two animals that were not pregnant, two that died, and two that aborted, resulting in a lower number of litters at this dose (16) compared to controls (21). When examined on a per litter basis, there were no effects of treatment on the mean numbers live fetuses, early resorptions, or late resorptions. Furthermore, fetal body weights, sex ratio, and post-implantation losses in the treated groups were comparable to controls.

There were no treatment-related external, visceral, or skeletal malformations or variations.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day (limit dose).

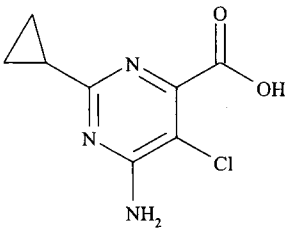
This study is classified **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.3700b; OECD 414) for a developmental toxicity study in rabbits.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Aminocyclopyrachlor

Description:	White solid
Lot #:	MAT28-009
Purity:	92.2% a.i.
Compound stability:	Stable in the vehicle for up to 4.25 hours at room temperature
CAS #:	858956-08-8
Structure:	

2. **Vehicle:** aqueous 0.5% (w/v) methylcellulose

3. **Test animals**

Species:	Rabbit
Strain:	New Zealand White
Age/ weight range at GD 0:	5.5 months; 2981-4355 g females
Source:	Covance Research Products, Inc. (Kalamazoo, MI)
Housing:	Individually in stainless steel cages suspended above ground corn cob bedding
Diet:	Certified Rabbit LabDiet® 5322 (PMI Nutrition International, LLC, St. Louis, MO), offered in gradual increments 3 times per day to a maximum of 150 g/day during the period prior to dosing, and then *ad libitum* for the remainder of the study
Water:	Reverse osmosis purified (onsite) tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18.5-19.2°C **Humidity:** 55.0-67.6% **Air changes:** 10/hr **Photoperiod:** 12 hours light : 12 hours dark
Acclimation period:	Approximately 4 to 6 days

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: August 7, 2007 End: August 31, 2007

2. **Mating:** Nulliparous time-mated females were received from the supplier on gestation day (GD) 1, 2, or 3. It was stated that the day that mating was confirmed was designated GD 0; however, the method for determining positive evidence of mating (e.g., presence of a vaginal plug and/or sperm in a vaginal smear) was not described.

3. **Animal assignment:** Presumed pregnant dams were randomly assigned, stratified by body weight, to the treatment groups shown in Table 1.

TABLE 1. Animal assignment [a]					
Dose (mg/kg/day)	0	100	300	500	1000
No. females	22	22	22	22	22

a Data were obtained from page 22 of MRID 47560015.

4. **Dose-selection rationale:** Dose levels for the current study were based on results of a previously conducted dose range-finding study (WIL-189192, Fleeman, 2008), included in Appendix B on pages 328-604 of the study report. The test substance was administered via daily oral gavage at a dose volume of 4 mL/kg to time-mated female New Zealand White rabbits (8/group) once daily on gestation day 7-28 at dosages of 0, 100, 300, 500 or 1000 mg/kg/day. There were no effects on clinical observations, body weight, body weight gain, or food consumption at any dose level. One abortion occurred in the 1000 mg/kg/day group. Post-implantation loss was increased at 1000 mg/kg/day (19.2%) compared to controls (4.2%), primarily due to early resorptions (15.5% treated vs 4.2% controls), resulting in a corresponding lower percent of viable fetuses at this dose (80.8%) compared to controls (95.8%). The increase in post-implantation loss was due primarily to 1 doe with only 2 viable fetuses. Mean fetal body weight was decreased by 11% at 1000 mg/kg/day compared to controls, but was primarily due to 1 litter that had a mean body weight of 31.1 g (versus an average of 43.1 g in controls). Collectively, these differences from the control group were not of sufficient magnitude to preclude selection of 1000 mg/kg/day as the highest dosage level for use in a definitive developmental toxicity study. Therefore, the dosage levels for the current study were 0, 100, 300, 500, and 1000 mg/kg/day.

5. **Dose preparation, administration, and analysis:** The dose formulations were prepared daily by weighing the appropriate amount of the test substance (corrected for purity by applying a correction factor of 1.08) for each formulation into a glass mortar, adding a small amount of the vehicle to each mortar, and grinding the test substance and vehicle with a pestle until a uniform mixture was obtained. The resulting mixture was quantitatively transferred into a calibrated glass container. Approximately 70% of the total volume of the vehicle was added to each container, and the suspension was mixed to a uniform consistency using a magnetic stirrer. Vehicle was then added to each container to bring the formulations to the calibration mark. Dose formulations were maintained at room temperature for no more than 5 hours from the completion of preparation to the completion of dosing. The test substance formulations were stirred for at least 30 minutes prior to dosing and continuously throughout use. The pH was measured for the formulations prepared on 7 August 2007, with measurements for the 0, 25, 75, 125, and 250 mg/ml formulations of 8.41, 3.48, 3.45, 3.45, and 3.70. The dose suspensions were administered daily via oral gavage from GD 7-28 in a dose volume of 4 mL/kg body weight, based on the most recent body weight. Prior to the initiation of dose administration, duplicate samples for homogeneity determination were collected from the top, middle, and bottom strata of the 25 and 250 mg/mL dosing formulations. Furthermore, it was stated that the test substance formulations were visually inspected by the deputy director on the first day of treatment, and were found to be visibly homogeneous and acceptable for dose administration. Formulations were mixed using a magnetic stirrer for at least 30 minutes prior to sampling. Duplicate samples for concentration analyses were collected from the middle strata of each dosing formulation

(including the vehicle formulation administered to the control group) prepared on the first, tenth, and last day of test substance administration. Stability of the test substance was established in the range-finding study (WIL-189192, Fleeman, 2008) for 4.25 hours at room temperature at a concentration range of 25 to 250 mg/mL (equivalent to doses of 100 and 1000 mg/kg/day, respectively).

Results

Homogeneity: 1.7-3.3% RSD; 99.8-101% of nominal (calculated by reviewers. The RSD is the relative standard deviation, which is the absolute value of the coefficient of variation).

Stability (% initial): 100-101%

Concentration (mean % nominal): 96.9-102% of nominal

It was stated that a small number of black particulates were noted in the dosing suspensions on August 25 and 29, 2007 during the preparation of dosing formulations and that the black particles were also present in the bulk compound. However, the analytical data indicate that the test substance was homogeneous and stable in the suspensions and that the variation between the nominal and actual dosages to the animals was acceptable.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** Cage-side checks for mortality and moribundity were conducted twice daily (once in the morning and once in the afternoon). Individual detailed clinical observations were conducted daily from the day of receipt through euthanasia. Additionally during the treatment period, the rabbits were observed for clinical signs of toxicity approximately one hour post-dosing. Each animal was weighed on GD 0 (by the supplier), GD 4 and daily from GD 7-29. Mean maternal body weight changes and absolute (g/animal/day) and relative to body weight (g/kg/day) food consumption were reported for the corresponding intervals between body weight measurements and additionally for GD 7-10, 10-14, 14-17, 17-20, 20-24, 24-29, and for the overall (GD 7-29) treatment period. When body weights and food consumption could not be determined for an animal during a given interval, group mean values were calculated for that interval using available data. On GD 29, all surviving rabbits were euthanized by intravenous injection of sodium pentobarbital via the marginal ear vein. Gravid uterine weight was collected and net body weight (GD 29 body weight exclusive of the weight of the uterus and contents) and net body weight change (the GD 7-29 body weight change exclusive of the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy. A gross necropsy was performed on each doe, and the liver and kidneys were removed and weighed. The kidneys, liver, stomach, and any gross lesions were preserved in 10% neutral-buffered formalin for possible future histopathology examination. The uterus and ovaries were removed via cesarean section. The numbers of corpora lutea in each ovary were counted. The uterine contents were examined, and the numbers and relative positions of implantations, early resorptions, late resorptions, live fetuses, and dead fetuses were recorded. Uteri without visible implantations were immersed in 10% aqueous

ammonium sulfide to detect very early resorptions (Salewski, 1964). Post-mortem examinations for unscheduled deaths (i.e., aborted and euthanized or found dead) were similar to those euthanized on GD 29, with the exception that organ weights were not obtained, and only gross lesions were preserved.

2. **Fetal evaluations:** Each live fetus was weighed, examined for external abnormalities, and euthanized via hypothermia (followed by intrathoracic injection of sodium pentobarbital if necessary). Each viable fetus was then subjected to a visceral examination using a modification of the Stuckhardt and Poppe fresh dissection technique to include the heart and major blood vessels (Stuckhardt and Poppe, 1984). The sex of each fetus was determined by internal examination. Fetal kidneys were examined and graded for renal papillae development (Woo and Hoar, 1972). Heads from all fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethanol. Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S (Dawson, 1926). External, visceral and skeletal findings were recorded as developmental variations (alterations in anatomic structure that are considered to have no significant biological effect on animal health or body conformity and/or occur at high incidence, representing slight deviations from normal) or malformations (those structural anomalies that alter general body conformity, disrupt or interfere with normal body function, or may be incompatible with life).

D. DATA ANALYSIS

1. **Statistical analyses:** The following statistical analyses were performed. Significance was denoted at $p<0.05$ and $p<0.01$. Statistical methods were considered appropriate.

Parameter	Method of Statistical Analysis
Maternal body weight Maternal body weight change Maternal food consumption Gravid uterus weight Numbers of: Corpora lutea Implantations Live fetuses Fetal body weights Organ weights	One-way analysis of variance (ANOVA) to detect differences among groups. If ANOVA was significant ($p<0.05$), Dunnett's test was used for pair-wise comparisons of the treated groups with the controls.
Mean litter proportions (percent per litter) of: Live fetuses Dead fetuses Early resorptions Late resorptions Total resorptions Pre-implantation loss Post-implantation loss Sex ratio External, visceral, and skeletal malformations External, visceral, and skeletal variations	Kruskal-Wallis non-parametric ANOVA to detect differences among groups. If Kruskal-Wallis was significant ($p<0.05$), Dunn's test was used for pair-wise comparisons of the treated groups with the controls.

2. **Indices:** The following indices were calculated from the cesarean section record of each animal in the study:

   Pre-implantation loss (%) = (# corpora lutea – # implantations)/ # corpora lutea x 100

   Post-implantation loss (%) = (# dead fetuses + resorptions)/ # implantations x 100

3. **Historical control data:** Historical control data were provided for cesarean parameters and fetal external, visceral, and skeletal examinations. These data comprised a total of 47 studies (one summary of 39 studies and another of 8 studies) conducted from 2001-2007.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical signs:** In the 1000 mg/kg/day group, two females were found dead and two females aborted during the course of the study. Female No. 51135 was found dead on GD 13. Increased respiration was observed at the 1 hour post-dose on GD 7 in this animal, and rales and decreased defecation were noted at the daily examinations during GD 9-13. This female had a body weight loss of 394 g from the first day of dosing (GD 7) and consumed ≤14 g of food per day for 4 of the 5 days prior to death. Although a cause of death was not determined at necropsy, this mortality was considered to be treatment-related. The other mortality observed in this group (Animal No. 51108 found dead on GD 27 with no

significant clinical observations noted prior to death) was due to gavage error and not treatment. In addition, female nos. 51092 and 51155 in this group aborted on GD 20 and 26, respectively. Clinical findings noted for both females included decreased defecation several days prior to abortion and red material in the cage pan or anogenital area on the day of abortion. These females exhibited treatment-related body weight losses of 636 g to 643 g (approximately 19% to 17%) from the first day of dosing (GD 7) through the day of abortion. These maternal effects corresponded with reduced food consumption, generally ≤17 g/day from GD 13 and 17 for the same respective females through the day of abortion.

One female in the 100 mg/kg/day group was found dead and 1 female in the 300 mg/kg/day group aborted during the course of the study. Female no. 51175 in the 300 mg/kg/day group aborted 2 live fetuses and 7 dead fetuses on GD 28. Clinical findings noted for this female prior to abortion included 11 occurrences of decreased defecation and 3 occurrences of soft stool; this female consumed ≤18 of food per day between GD 18-19 and 27-28, but had no notable effects on body weight gain. In addition, female no. 51096 in the 100 mg/kg/day group was found dead on GD 22; this female had no significant clinical observations noted prior to death. The death at 100 mg/kg/day and the abortion at 300 mg/kg/day were not considered treatment-related because they were unrelated to dose. All other maternal animals survived until scheduled termination.

Increased incidence of soft stool was noted in the 500 and 1000 mg/kg/day groups beginning on GD 18 and continuing throughout the remainder of the study. In addition, an increased incidence of hair loss around the limbs and urogenital, abdominal, and inguinal areas was observed in the 1000 mg/kg/day group. There were no other dose or test substance-related clinical findings.

2. **Body weights and body weight gains:** Selected maternal body weight and body weight gain data are presented in Table 2. Body weights of the treated groups were comparable to controls throughout the study. At 1000 mg/kg/day, a significant ($p<0.05$) body weight loss of 9 g was observed during GD 14-17 compared to a gain of 70 g in the control group. Additionally in this group, non-significant (NS) decreases in body weight gains were noted for GD 17-20 (13 g vs 44 g controls) and GD 20-24 (13 g vs 39 g controls). For GD 24-29, a body weight loss (NS) of 35 g was observed at 1000 mg/kg/day compared to a gain of 15 g in controls.
The only finding at 500 mg/kg/day was a body weight loss of 56 g for GD 24-29 compared to a gain of 15 g in controls. Body weight gains in the 100 and 300 mg/kg/day groups were comparable to controls throughout the study. Gravid uterine weights and corrected (net) body weight gain (GD 7-29) in the treated groups were comparable to controls.

TABLE 2. Mean (±SD) maternal body weights and body weight gains (g) [a]

Parameter/Interval	Dose in mg/kg bw/day (# of Dams)				
	0 (21)	100 (20-21)	300 (22)	500 (22)	1000 (16-20)
Body weights					
GD 0	3573±257.9	3563±246.2	3507±228.9	3508±226.9	3561±276.6
GD 7	3640±243.9	3656±255.8	3613±184.9	3595±207.0	3619±264.9
GD 29	3913±298.1	3862±363.5	3903±243.5	3768±244.5	3800±373.1
Corrected body weight GD 29 [b]	3404.4±258.6	3387.7±332.6	3393.1±219.9	3286.7±208.3	3337.2±335.7
Body weight gains					
Pre-treatment: GD 0-4	-62±48.6	-28±91.0	-41±90.1	-46±60.5	-63±82.8
GD 4-7	129±68.3	121±53.4	147±77.7	132±78.2	121±91.8
Treatment: GD 7-10	38±53.4	39±66.6	70±59.6	36±45.8	36±92.2
GD 10-14	66±56.5	70±39.0	67±72.9	66±42.1	52±67.6
GD 14-17	70±63.4	68±41.0	34±93.5	36±85.8	-9±79.0**
GD 17-20	44±50.3	45±34.9	41±70.7	42±54.7	13±89.9 (↓70)
GD 20-24	39±102.8	21±87.8	63±83.0	49±98.0	13±88.3 (↓67)
GD 24-29	15±111.9	-20±124.5	0±119.5	-56±174.0	-35±185.1
Overall BW gain: GD 7-29	273±175.0	218±201.1	292±161.6	173±190.9 (↓37)	166±234.7 (↓39)
Gravid uterine weight (g)	508.3±87.0	474.4±79.8	510.3±84.1	481.0±86.4 (↓5.3)	462.9±61.4 (↓9.1)
Corrected BW gain: GD 7-29 [c]	-235.2±165.9	-256.4±182.4	-218.6±170.6	-307.8±165.4	-297.2±206.1

a Data were obtained from Tables 4 through 6 on pages 43-55 of the study report. Percent differences from controls, calculated by the reviewers, are included in parentheses.
b Corrected (net) body weight = terminal body weight – gravid uterine weight
c Corrected body weight gain = net body weight on GD 29 – body weight on GD 7
** Significantly different from the controls at $p<0.01$

3. **Food consumption:** At 1000 mg/kg/day, absolute and relative to body weight food consumption was decreased ($p<0.05$) by 27-31% for GD 14-15, 15-16, and 14-17 compared to controls (Table 3). In spite of the significant decreases between GD 14 and 17 at 1000 mg/kg/day, the mean food consumption for the overall (GD 7-29) treatment period at this dose was slightly decreased (↓7-8%) and not statistically significant. There were no significant decreases in food consumption at 100 or 500 mg/kg/day compared to controls, although a minor decrease (NS) of 8% compared to controls was noted at 500 mg/kg/day for the overall treatment period. At 300 mg/kg/day, food consumption was significantly increased ($p<0.05$) by 22-24% for GD 11-12 and 12-13 compared to controls. However, the increases at 300 mg/kg/day were not dose-dependent or adverse and were considered unrelated to treatment.

TABLE 3. Mean (±SD) maternal food consumption (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)				
	0 (21)	100 (20-21)	300 (22)	500 (22)	1000 (16-20)
Absolute (g/animal/day)					
GD 11-12	151 ± 33.7	170 ± 33.9	185 ± 34.9** (↑23)	172 ± 28.6	160 ± 44.6
GD 12-13	136 ± 35.4	150 ± 31.2	168 ± 39.2* (↑24)	135 ± 40.7	135 ± 47.2
GD 14-15	149 ± 56.3	166 ± 54.6	138 ± 54.2	113 ± 55.7	103 ± 48.7* (↓31)
GD 15-16	156 ± 46.5	160 ± 37.2	141 ± 59.5	123 ± 60.8	108 ± 44.6* (↓31)
GD 14-17	156 ± 43.9	165 ± 34.9	157 ± 74.9	120 ± 54.8	112 ± 45.0* (↓28)
GD 7-29	135 ± 24.4	135 ± 28.0	139 ± 27.4	124 ± 22.3 (↓8)	126 ± 21.3 (↓7)
Relative to body weight (g/kg bw/day)					
GD 11-12	41 ± 9.4	46 ± 7.8	50 ± 9.5** (↑22)	47 ± 7.6	44 ± 12.6
GD 12-13	37 ± 9.7	40 ± 7.0	45 ± 10.3* (↑22)	37 ± 10.3	36 ± 12.6
GD 14-15	40 ± 15.2	44 ± 13.5	36 ± 14.1	30 ± 14.4	28 ± 12.9* (↓30)
GD 15-16	41 ± 12.7	42 ± 8.9	37 ± 15.5	33 ± 15.6	29 ± 11.1* (↓29)
GD 14-17	41 ± 11.9	43 ± 8.0	41 ± 18.9	32 ± 13.8	30 ± 11.5* (↓27)
GD 7-29	36 ± 6.1	35 ± 6.0	37 ± 6.6	33 ± 5.3 (↓8)	33 ± 4.8 (↓8)

a Data were obtained from Tables 7 and 8 on pages 57, 61, 63, and 67 of the study report. Percent differences from controls, calculated by the reviewers, are included in parentheses. Data for dosing intervals with significant differences and over the entire dosing interval (GD 7-29) are provided.

* Significantly different from the controls at $p<0.05$

** Significantly different from the controls at $p<0.01$

4. **Gross pathology:** The following gross findings observed at 1000 mg/kg/day (# affected compared to 0 controls) were related to the previously described treatment-related deaths (one) and abortions (two): (i) red material around the nasal area, accessory spleen, and white precipitate in the abdominal cavity adjacent to the right kidney in the animal that died on GD 13 (#51135), (ii) red skin matting around the urogenital region and dark red contents in the vagina in the animal that aborted GD 20 (#51092); (iii) red skin matting around the urogenital region in the animal that aborted GD 26.

   The following gross findings were observed in the two deaths considered unrelated to treatment. Red fluid was found in the thoracic cavity and esophageal perforation was observed in the 1000 mg/kg/day animal that died on GD 27 due to gavage error (#51108). An accessory spleen, cystic oviducts, 7 normally developing implantations, and 1 early resorption in utero was observed for the 100 mg/kg/day dam that died on GD 22.

   No internal abnormalities were found for the 300 mg/kg/day animal that survived to terminal kill but aborted on GD 28 (#51175).

   For the animals that survived to scheduled necropsy on GD 29, no test substance-related macroscopic findings were observed at doses of 100, 300, 500, and 1000 mg/kg/day. One female in both the control and 100 mg/kg/day groups and 2 females in the 1000 mg/kg/day group were determined to be non-gravid. Mean liver and kidney weights were not affected by test substance administration.

5. **Cesarean section data:** Summary data from the cesarean sections are presented in Table 4. There were no complete litter resorptions or dead fetuses. At 1000 mg/kg/day, there were two animals that were not pregnant, two that died, and two that aborted, resulting in a lower

number of litters at this dose (16) compared to controls (21). When examined on a per litter basis, there were no effects of treatment on the mean numbers live fetuses, early resorptions, or late resorptions. Furthermore, fetal body weights, sex ratio, and post-implantation losses in the treated groups were comparable to controls.

TABLE 4. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)				
	0	100	300	500	1000
# Animals assigned (mated)	22	22	22	22	22
# Animals pregnant	21	21	22	22	20
Pregnancy rate (%) [b]	95.5	95.5	100	100	90.9
# Nonpregnant	1	1	0	0	2
Maternal wastage					
No. died	0	1	0	0	2
No. died pregnant	0	1	0	0	2
No. died nonpregnant	0	0	0	0	0
No. aborted	0	0	1	0	2
No. premature delivery	0	0	0	0	0
Total no. corpora lutea	217	204	217	232	165
Corpora lutea/dam	10.3 ± 2.20	10.2 ± 1.91	10.3 ± 1.68	10.5 ± 1.57	10.3 ± 2.18
Total no. implantations	203	179	200	219	149
(Implantations/dam)	9.7 ± 2.06	9.0 ± 2.09	9.5 ± 1.72	10.0 ± 1.73	9.3 ± 1.49
Total no. litters	21	20	21	22	16
Total no. live fetuses	195	167	193	209	138
(Live fetuses/dam)	9.3 ± 1.87	8.4 ± 2.16	9.2 ± 1.66	9.5 ± 1.82	8.6 ± 1.50
Total no. dead fetuses	0	0	0	0	0
(Dead fetuses/dam)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Total no. resorptions	8	12	7	10	11
Early	4	9	4	6	2
Late	4	3	3	4	9
Resorptions/dam	0.4 ± 0.67	0.6 ± 0.82	0.3 ± 0.80	0.5 ± 0.67	0.7 ± 1.20
Early	0.2 ± 0.51	0.5 ± 0.76	0.2 ± 0.68	0.3 ± 0.46	0.1 ± 0.34
Late	0.2 ± 0.51	0.2 ± 0.37	0.1 ± 0.48	0.2 ± 0.50	0.6 ± 1.15
Complete litter resorptions	0	0	0	0	0
Mean fetal weight (g), males	40.0 ± 4.06	41.4 ± 5.78	40.8 ± 5.64	37.3 ± 5.22	38.0 ± 5.62
females	39.5 ± 4.20	40.8 ± 5.78	40.1 ± 5.01	36.2 ± 4.99	37.8 ± 5.58
Sex ratio (% male)	41.5 ± 12.51	49.3 ± 16.99	55.5 ± 13.20	46.8 ± 14.33	45.0 ± 21.33
Pre-implantation loss (%)	6.2 ± 7.11	12.2 ± 12.40	7.7 ± 8.83	5.5 ± 8.90	8.4 ± 9.83
Post-implantation loss (%)	3.6 ± 6.34	6.7 ± 9.57	3.2 ± 7.62	4.6 ± 6.63	6.9 ± 11.52

a Data were obtained from Tables 1, 2, 11, and 12 on pages 39, 40, and 71-74 of the study report.
b Calculated by the reviewers from data presented in this table.

B. DEVELOPMENTAL TOXICITY

1. **Malformations:** All external, visceral, and skeletal malformations are presented in Table 5. No external or visceral malformations were observed at 1000 mg/kg/day. Upon external examination, a single fetus in the 500 mg/kg/day group was found to have multiple malformations of the head, including microcephaly, proboscis-like nose, mandibular and maxillary micrognathia, bilateral anophthamia, and astomia. These external malformations

were confirmed at the visceral and skeletal examinations. There were no treatment-related skeletal malformations. Vertebral anomaly with or without associated rib anomaly was noted in a single fetus each at 300, 500, and 1000 mg/kg/day compared to 0 concurrent controls. However, this finding was also noted in the historical control data. Additionally at 1000 mg/kg/day, two fetuses had only 6 cervical vertebrae present. Although this malformation was not reported in the historical control data, it was considered incidental to treatment because it was minimal in incidence and occurred only in a single litter. There were no other malformations.

TABLE 5. Fetal malformations [# fetuses (litters) affected] [a]

Observation	Dose (mg/kg bw/day)				
	0	100	300	500	1000
No. fetuses (litters) examined	195 (21)	167 (20)	193 (21)	209 (22)	138 (16)
External malformations					
Microcephaly	---	---	---	1 (1) [b]	---
Proboscis-like nose	---	---	---	1 (1) [b]	---
Mandibular micrognathia	---	---	---	1 (1) [b]	---
Maxillary micrognathia	---	---	---	1 (1) [b]	---
Microphthalmia and/or anopthalmia [c]	---	---	1 (1)	1 (1) [b]	---
Astomia	---	---	---	1 (1) [b]	---
Macroglossia	1 (1)	1 (1)	---	---	---
Carpal and/or tarsal flexure	3 (2)	---	---	---	---
Paw hyperflexion	2 (1)	---	---	---	---
Total no. with external malformations	4 (3)	1 (1)	1 (1)	1 (1)	---
Visceral malformations					
Hydrocephaly	---	---	---	1 (1) [b]	---
Retroesophageal aortic arch	---	1 (1)	---	---	---
Persistent trucus arteriosus	1 (1)	---	---	---	---
Total no. with visceral malformations	1 (1)	1 (1)	---	1 (1)	---
Skeletal malformations					
Vertebral anomaly with or without associated rib anomaly	---	---	1 (1)	1 (1)	1 (1)
Only 6 cervical vertebrae present	---	---	---	---	2 (1)
Costal cartilage anomaly	---	---	2 (1)	---	---
Centra anomaly	---	---	1 (1)	---	---
Rib anomaly	1 (1)	---	---	---	---
Total no. with skeletal malformations	1 (1)	---	4 (3)	1 (1)	3 (2)
Total no. with malformations (combined)	6 (5)	2 (2)	5 (3)	2 (2)	3 (2)

a Data were obtained from Table 13 on page 75 and Table A13 on page 289 of the study report.
b Findings denoted by this superscript were observed in the same fetus (#6 from Doe #51104).
c Individual data for this animal denote the malformation as bilateral anopthalmia.
--- No animals affected (i.e., zero incidence)

2. **Variations:** Selected fetal variations are presented in Table 6. In order to make comparisons to historical controls, data were presented as mean percent fetal incidence (% litter incidence not available). There were no external variations. An increased incidence of extra papillary muscle in the heart was found in all treated groups (4.4-7.6% fetuses) compared to concurrent (4.2%) and historical (4.0%) controls. However, because these increases were minor, not statistically significant, and did not show a clear dose-response, they were considered unrelated to treatment. Accessory spleen was commonly observed in all treated groups (7.8-14.1%), in addition to the concurrent controls (12.9%). However, the incidences of this

variation were unrelated to dose and were within the range of historical controls (3.5-33.2% fetuses). Incidences of extra site of ossification ventral to cervical centrum #2 were noted at 100 mg/kg/day (0.4% fetuses) and 1000 mg/kg/day (4.2% fetuses) compared to 0 concurrent controls. Although the mean incidence of this variation at 1000 mg/kg/day (4.2%) exceeded the range of historical controls (0.0-0.7%), the increased incidence was considered unrelated to treatment because it was minor and not statistically significant. Additionally, vertebral centra not fully ossified was observed at 1000 mg/kg/day (0.6%) compared to 0 concurrent or historical controls; however, this finding was considered incidental because it occurred in a single fetus. Incidences of all other visceral and skeletal variations were unrelated to dose.

TABLE 6. Selected fetal variations [mean ± SD % fetuses affected] [a]

Observation	Dose (mg/kg bw/day)					
	0	100	300	500	1000	Historical Controls [b]
No. fetuses (litters) examined	195 (21)	167 (20)	193 (21)	209 (22)	138 (16)	
External variations						
Total % with external variations	---	---	---	---	---	NA
Visceral variations						
Heart – extra papillary muscle	4.2±8.57	4.4±7.96	6.3±12.88	5.4±8.46	7.6±7.83	0.0-4.0
Accessory spleen	12.9±13.50	14.1±26.28	8.7±11.48	7.8±10.92	7.9±11.23	3.5-33.2
Total % with visceral variations	29.8±19.86	27.1±26.54	26.2±21.21	20.7±24.63	24.3±20.61	NA
Skeletal variations						
Extra site of ossification ventral to cervical centrum #2	---	0.4±1.86	---	---	4.2±12.91	0.0-0.7
Vertebral centra not fully ossified	---	---	---	---	0.6±2.27	NR
13th full rib(s)	23.9±23.22	34.8±20.69	36.7±31.80	40.9±33.45	36.5±28.78	21.8-58.6
13th rudimentary rib(s)	18.0±12.00	22.0±16.76	14.4±17.59	14.6±13.47	24.0±15.92	8.1-29.7
27 presacral vertebrae	6.3±10.61	10.1±12.37	4.3±8.60	13.5±20.92	10.4±24.00	7.5-33.1
Total % with skeletal variations	55.7±23.35	66.7±22.84	67.4±24.62	66.6±29.16	68.6±19.55	NA
Total % with variations (combined)	71.0±17.55	74.9±19.19	78.2±21.16	73.4±25.81	78.1±12.78	NA

a Data were obtained from Table 16 on pages 84-88 of the study report.
b Historical control data were obtained from Appendix G on pages 655 and 656 of the study report. Minimum and maximum fetal incidence are reported.
--- No animals affected (i.e., zero incidence)
NA Not applicable
NR Not reported

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATOR'S CONCLUSIONS:** There was a treatment-related death and abortion at 1000 mg/kg/day. Body weight gains were decreased beginning on GD 24 at 500 mg/kg/day and on GD 14 at 1000 mg/kg/day and continuing generally throughout the remainder of the study. Therefore, it was concluded that the maternal LOAEL was 500 mg/kg/day based on decreased body weight gains and food consumption. In the absence of developmental toxicity at any dose level, the LOAEL for developmental toxicity was not observed.

B. REVIEWER COMMENTS

1. **Maternal toxicity:** At 1000 mg/kg/day, one animal (No. 51135) was found dead on GD 13. Increased respiration was observed at the 1 hour post-dose on GD 7 in this animal, and rales and decreased defecation were noted at the daily examinations during GD 9-13. This female had a body weight loss of 394 g from the first day of dosing (GD 7) and consumed ≤14 g of food per day for 4 of the 5 days prior to death. Although a cause of death was not determined at necropsy, this mortality was considered to be treatment-related.

   Additionally at this dose, female nos. 51092 and 51155 aborted on GD 20 and 26, respectively. Clinical findings noted for both females included decreased defecation several days prior to abortion and red material in the cage pan or anogenital area on the day of abortion. These females exhibited treatment-related body weight losses of 636 g to 643 g (approximately 19% to 17%) from the first day of dosing (GD 7) through the day of abortion. These maternal effects corresponded with reduced food consumption, generally ≤17 g/day from GD 13 and 17 for the same respective females through the day of abortion. It was stated that increased risk of abortion has been associated with decreased food consumption and greater than 10% body weight loss in rabbits (Cappon *et al.*, 2005)[1]. Therefore, the abortions were considered to be treatment-related but likely secondary to the decreased food consumption. There were no other treatment-related deaths or abortions. Aside from the above-mentioned clinical signs associated with mortality and abortion, no treatment-related clinical signs were observed.

   Body weights of the treated groups were comparable to controls throughout the study. At 1000 mg/kg/day, a significant ($p<0.05$) body weight loss of 9 g was observed during GD 14-17 compared to a gain of 70 g in the control group. Additionally in this group, non-significant (NS) decreases in body weight gains were noted for GD 17-20 (13 g vs 44 g controls) and GD 20-24 (13 g vs 39 g controls). For GD 24-29, a body weight loss (NS) of 35 g was observed at 1000 mg/kg/day compared to a gain of 15 g in controls. The only finding at 500 mg/kg/day was a body weight loss of 56 g for GD 24-29 compared to a gain of 15 g in controls. Gravid uterine weights and corrected (net) body weight gain (GD 7-29) in the treated groups were comparable to controls.

   At 1000 mg/kg/day, absolute and relative to body weight food consumption was decreased ($p<0.05$) by 27-31% for GD 14-15, 15-16, and 14-17 compared to controls. In spite of the significant decreases between GD 14 and 17 at 1000 mg/kg/day, the mean food consumption for the overall (GD 7-29) treatment period at this dose was slightly decreased (↓7-8%) and not statistically significant. Although a minor decrease of 8% at 500 mg/kg/day was noted for the overall treatment period, this decrease was not considered adverse because it did not impact body weights or body weight gains.

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1 Cappon, G.D., Fleeman, T.L., Chapin, R.E., and Hurtt, M.E. Effect of Feed Restriction During Organogenesis on Embryo-Fetal Development in Rabbit. *Birth Defects Research (Part B)*, **2005**, 74, 424-430.

The only treatment-related gross findings occurred at 1000 mg/kg/day, and were related to the previously described treatment-related deaths and abortions. These findings included red skin matting around the urogenital or nasal areas, dark red contents in the vagina, and white precipitate in the abdominal soft tissue.

The investigator's conclusions differed from the reviewer's conclusions regarding the appropriate maternal NOAEL. The investigator considered the (non-significant) reductions in body weight gain and food consumption a biologically significant effect of treatment and set the maternal LOAEL at 500 mg/kg/day. The reviewer did not consider the reductions in body weight and food consumption of biological significance and set the maternal LOAEL at 1000 mg/kg/day based on decreased body weight and food consumption and mortality and abortion. Both reviewers agreed that no developmental LOAEL was observed.

The reductions in food consumption were statistically significant only in the 1000 mg/kg/day group. These reductions were consistently about 30% after GD 14-15 through the end of the study. Decreases in body weight gain were statistically significant only at GD 14-17, although the decreases throughout the remainder of the study are probably of biological significance. This consistent relationship between decreased food consumption and decreased body weight gain were not observed in the 500 mg/kg/day group. In this group, no significant differences in body weight gain were observed and there were no significant decreases in food consumption.

Therefore, the maternal LOAEL is 1000 mg/kg/day based on decreased body weight gain and food consumption and abortion and mortality.

The maternal LOAEL is 1000 mg/kg/day (limit dose) based on incidences of mortality and abortion and decreased body weight gains and food consumption. The maternal NOAEL is 500 mg/kg/day.

2. **Developmental toxicity**

a. **Deaths/resorptions:** At 1000 mg/kg/day, there were two animals that were not pregnant, two that died, and two that aborted, resulting in a lower number of litters at this dose (16) compared to controls (21). When examined on a per litter basis, there were no effects of treatment on the mean numbers live fetuses, early resorptions, or late resorptions. Furthermore, fetal body weights, sex ratio, and post-implantation losses in the treated groups were comparable to controls.

b. **Altered growth:** There was no evidence of altered growth or development. Fetal body weights in the treated groups were comparable to controls. There were no treatment-related effects on ossification of the skeleton.

c. **Developmental variations:** There were no treatment-related external, visceral, or skeletal variations.

d. **Malformations:** There were no treatment-related external, visceral or skeletal

malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 1000 mg/kg/day (limit dose).

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.3700a; OECD 414) for a developmental toxicity study in rabbit

C. **STUDY DEFICIENCIES:** No deficiencies were observed.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.3800 [§83-4]; Multigeneration Reproduction Study in Rats

Work Assignment No. 5-1-209 H (MRID 47575101)

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Risk Assessment Branch III, Health Effects Division (7509P) **Date:** 10/15/2009

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - [rat]; OPPTS 870.3800 [§83-4]; OECD 416.

PC CODES: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (90.9-92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Lewis, J.M. (2008) DPX-MAT28 technical: multi-generation reproduction study in rats. E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-22032, October 15, 2008. MRID 47575101. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 47575101), DPX-MAT28 (Aminocyclopyrachlor; 90.9-92.2% a.i.; Batch Nos. DPX-MAT28-009 and DPX-MAT28-010) was administered in the diet to 28 Sprague Dawley (Crl:CD[SD]) rats/sex/dose group at dietary levels of 0, 500, 1500, 5000, or 17,000 ppm (equivalent to 0/0, 36/41, 109/125, 363/416, and 1285/1454 mg/kg bw/day in males/females during pre-mating) for two successive generations with one litter per generation. The P (parental) generation animals were fed the test diets for at least ten weeks prior to mating to produce the F1 litters. The F1 litters were culled on post-natal day (PND) 4 to eight pups/litter (four/sex where possible). On PND 21, one pup/sex/litter (where possible) was selected and fed the same test diet concentration as the dam. These animals were fed the test diets for at least ten weeks prior to mating (at 13 weeks of age) to produce the F2 litters.

No treatment-related effects were observed on mortality, clinical signs, or macroscopic or microscopic findings.

Over the pre-mating period in P and F1 males, body weights were significantly ($p \leq 0.05$) decreased (↓6-15%) at 5000 ppm and above, as were body weight gains (↓7-17%). Food efficiency was decreased (↓8-15%) at 1500 ppm and above. For P and F1 females over this period, body weights were decreased (↓6-14%) at 17,000 ppm. Food efficiency and body weight gain were decreased for P females only at 17,000 ppm (↓12-16%).

Over the period of gestation in P and F1 females, body weight and body weight gain was significantly decreased for P females only at 17,000 ppm (↓8-12%). Food consumption and food efficiency were also decreased for these animals (↓10% and ↓20%, respectively). There were no significant effects of the test substance on these parameters in F1 females. Over the latctation period, body weights were transiently but significantly decreased (↓7-9%) in P females at 17,000 ppm on LD 0-7. Decreases in body weights of F1 females were not statistically significant.

Terminal body weights were significantly ($p \leq 0.05$) decreased at 17,000 ppm for P males and females (↓6-7%) F1 males (↓16%). At 5,000 ppm, terminal body weight was decreased (↓9%) for F1 males only. There were significant decreases (↓4-5%) in absolute (but not relative) brain weights for P and F1 females at 17,000 ppm. At 5,000 ppm, decreases in absolute brain weights were observed for P females only (↓4%). There were no correlating microscopic findings.

The LOAEL for parental toxicity is 17, 000 ppm (equivalent to 1285/1454 mg/kg in males/females), based on decreased body weights in parental males and females. The NOAEL is 5000 ppm (equivalent to 363/416 mg/kg in males/females).

No treatment-related effects on litter parameters were noted for the F1 or F2 generations. Specifically, there were no treatment-related effects on survival indices, clinical signs, sex ratio, attainment of developmental landmarks, or gross or microscopic pathology.

At 17,000 ppm, body weights were significantly ($p \leq 0.05$) decreased at PND 21 in the F1 (↓15%) and F2 pups (↓8%). Body weights in the F1 pups were consistently decreased at PND 0, 4, 7, 14, and 21 at this dose, whereas body weights were decreased in F2 pups only at PND 21. There were no statistically significant decreases in body weight gain. In weanlings, terminal body weights were significantly decreased in both generations for both sexes (F1 males, F1 females, F2 males, F2 females) at 17,000 ppm (↓9-14%).

The LOAEL for offspring toxicity is 17,000 ppm (equivalent to 1285/1454 mg/kg in males/females), based on decreased body weights in the F1 and F2 pups. The NOAEL is 5000 ppm (equivalent to 363/416 mg/kg in males/females).

No treatment-related differences were observed in estrus cycle length or cyclicity, sperm parameters, numbers of primordial and pre-antral follicles, mating, fertility, pre-coital interval length, gestation length, number of implantation sites, or post-implantation loss, or reproductive failures. Thus reproductive performance of P and F1 animals was not affected by treatment.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 17,000 ppm (equivalent to 1285/1454 mg/kg in males/females).

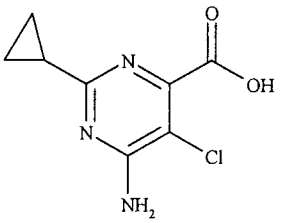
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28 Technical (Aminocyclopyrachlor)

Description:	White solid
Batch Nos.:	DPX-MAT28-009 and DPX-MAT28-010
Purity:	92.2% a.i. (Batch -009) and 90.9% a.i. (Batch -010)
Compound stability:	Stable in the diet for up to 14 days when stored at room temperature or 21 days when stored refrigerated
CAS # of TGAI:	858956-08-8
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Sprague Dawley (Crl:CD[SD])
Age at study initiation:	Approximately 56 days
Body weight at study initiation:	247-306 g males 167-219 g females
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Rats were housed individually in stainless steel, wire-mesh cages suspended above cage board during pre-mating, with one female housed in the male's cage during mating. Mated females were housed individually in suspended, stainless steel, wire mesh cages during gestation; beginning on GD 20, mated females were housed in polycarbonate pans with bedding. Non-mated females were housed in polycarbonate pans with bedding beginning six days after the mating period.
Diet:	Powdered Certified Rodent LabDiet® 5002 (PMI Nutrition International, Inc., St. Louis, MO), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature** 18-26°C **Humidity** 30-70% **Air changes** Not provided **Light cycle** 12 hours light/12 hours dark
Acclimation period:	13 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: Start: 03/14/07 End: 07/02/08

2. Mating procedure: Each female was continually housed on a one-to-one basis with a randomly selected, non-sibling male of the same dose group in the male's cage. Cohabitation was continued until evidence of copulation was observed or until two weeks had elapsed. Each female was examined for signs of mating daily, as evidenced by the presence of a vaginal plug or sperm in the vaginal smear. The day evidence of copulation was confirmed was designated as gestation day (GD) 0. When copulation was confirmed,

the female was returned to her home cage. The cohabitation period ended on the morning of Day 15 of
co-housing.

3. **Study schedule:** The P generation animals were fed the test diets for at least ten weeks prior to mating to produce the F1 litters. Females were allowed to litter normally, and the day on which delivery was complete was designated as lactation day (LD) 0. Litters were standardized to eight pups (4/sex when possible) by random culling of pups on post-natal day (PND) 4; litters of 8 pups or fewer were not culled. At weaning on PND 21, offspring (one pup/sex/litter where possible) were randomly selected as the F1 parents and fed the test diets for ten weeks prior to mating to produce the F2 litters. For dose groups with insufficient litters and at the discretion of the study director, additional pups were chosen from randomly selected litters within the dose group to achieve the required group size.

4. **Animal assignment:** The P animals were ranked by their most recently recorded body weight and randomly assigned to the test groups shown in Table 1 using a computerized randomization procedure designed to produce a homogeneous distribution of body weights across groups.

TABLE 1. Animal assignment[a]

Test group	Dose (ppm)[b]	Animals/group			
		P Males	P Females	F1 Males	F1 Females
Control	0	28	28	28	28
Low	500	28	28	28	28
Mid-low	1500	28	28	28	28
Mid-high	5000	28	28	28	28
High	17,000	28	28	28	28

a Data were obtained from pages 18 and 20 of the study report.

b Exposure to the test substance was continuous throughout the study. Concentrations were adjusted for test substance purity (Although the analysis for Batch -010 indicated that the purity was 90.0%, all formulation calculations were based on 92.2% a.i.).

5. **Dose-selection rationale:** The doses were selected on the basis of a previously conducted one-generation reproductive toxicity study (DuPont-17315; not provided) in which animals were exposed to IN-KJM44, of which DPX-MAT28 is the major metabolite. In this study, it was stated that the P generation (10 Sprague Dawley rats/sex/dose) was exposed to dietary concentrations of 0, 600, 5000, and 17,000 ppm during a 4-week pre-mating period, 2-week mating period, gestation (3 weeks), and lactation (3 weeks). The F1 weanlings (1/sex/litter) randomly selected to continue the F1 generation were fed the same dietary concentrations as their parents until PND 60. Organs of the reproductive tract, adrenals, pituitary, and liver form P1 adults were weighed and examined microscopically. Previously identified target organs (pancreas, thyroid) were also examined microscopically for P and F1 adults and F1 weanlings. There were no effects on reproductive parameters at dietary concentrations up to 17,000 ppm. Adverse, treatment-related effects were observed on body weight and food consumption in the 17,000 ppm P females. The 17,000 ppm F1 pup body weights were decreased during lactation, continuing with recovery during the post-weaning period. There were no adverse effects observed during clinical pathology or during neurobehavioral evaluations in the P rats; and organ weights and gross and microscopic pathological findings were not affected by treatment at any dietary concentration. Based on this data, dietary concentrations of 0, 500, 1500, 5000, and 17,000 ppm were selected for this study.

6. **Test diet preparation and analysis:** For each dose level, an appropriate amount of the test substance was mixed directly with basal diet to yield the desired concentration. Control diets were mixed for a similar length of time. Diets formulated with Batch -009 were fed to the P generation on Days 1-27; Batch -010 was administered for the remainder of the study. Test diets were prepared at least biweekly and stored refrigerated until used. Homogeneity (top, middle, bottom) and concentration were verified in all dose levels of the first preparation and at approximately five months; concentration was also verified at approximately two and seven months. Stability was determined prior to study initiation in dietary formulations containing 300 and 18,000 ppm of the test compound following room temperature storage for 14 days or refrigerated storage for 21 days.

Results

Homogeneity (% CV): 0.7-5%

Stability (% of Day 0): 95.9-107% after room temperature storage for 14 days
99.4-117% after refrigerated storage for 21 days

Concentration (% of nominal): 92.4-110.6%, except 81.2% in the 17,000 ppm formulation in one of four samples

The analytical data indicate that the mixing procedure was adequate and that the variation between the target and actual dosage to the study animals was marginally acceptable.

7. **Dosage administration:** The test material was administered in the diet continuously throughout the study (i.e., P generation adults were fed the test diets *ad libitum* beginning ten weeks prior to mating, and the selected F1 adults were fed the same test diet concentrations as their parents beginning on PND 22).

C. OBSERVATIONS

1. **Parental animals:** All rats were examined at least daily for mortality and clinical signs of toxicity; detailed examinations were performed weekly. Body weights of the males were recorded prior to initiation of dosing, weekly throughout the study, and at termination. Body weight gains were reported for each weighing interval, and for Days 0-70, 70-119, and 0-119 in the P males and Days 0-70, 70-105, and 0-105 for the F1 males. Females were weighed prior to initiation of dosing, weekly throughout the pre-mating period, on GD 0, 7, 14, and 21, and on LD 0, 7, 14, and 21. Body weight gains were reported for each interval during pre-mating, gestation, and lactation, and for pre-mating Days 0-70, GD 0-21, and LD 0-21. Mean food consumption (g/animal/day) and food efficiency (body weight gain/food consumption) were reported weekly for both sexes during pre-mating and for Days 0-70; mean food consumption and efficiency were also reported for GD 0-7, 7-14, 14-21, and 0-21, and for LD 0-7, 7-14, and 0-14. Estrus cyclicity and duration were determined by examination of daily vaginal smears beginning three weeks prior to mating and continuing until mating was confirmed or the mating period ended. Sperm motility, enumeration

(cauda epididymis and testis), and morphology (% normal) were evaluated at necropsy in all males.

2. **Litter observations:** The following litter parameters (X) were recorded (Table 2):

TABLE 2. F1/F2 Litter Observations [a]

Observation	Time of observation (post-natal day)					
	Day 0	Day 4[b]	Day 4[c]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Number of dead pups	X	X		X	X	X
Pup weight	X	X		X	X	X
Sex of each pup (M/F)[d]	X	X	X	X	X	X
External alterations	X	X	X	X	X	X

a Data obtained from page 27 of the study report.
b Prior to standardization on PND 4
c After standardization on PND 4
d Although the number of pups of each sex was recorded on Days 0, 4, 7, 14, and 21, only Day 0 sex ratio was reported.

At each examination period (PND 0, 4, 7, 14, and 21), litters were examined for mortality and abnormal behavior and appearance. F1 females designated for mating were examined for vaginal patency daily beginning on PND 21 until achievement or PND 43; F1 males designated for mating were examined for preputial separation beginning on PND 35 until achievement or PND 55. Body weights were recorded on the day of achievement.

3. **Postmortem observations**

a. **Parental animals:** All surviving animals were euthanized by exsanguination under carbon dioxide anesthesia; all rats received a complete post-mortem examination. Males were killed after siring litters; nursing females were killed on LD 21; dams that had no live pups remaining during lactation were killed; and non-nursing females were killed approximately 5-6 weeks after the end of the mating period. Vaginal smears were collected from females on the day of necropsy and estrus cycle stage determined. The uteri of all cohabited females were examined for the presence and number of implantation sites (method not provided). The following tissues were collected for histological examination (X) and fixed in neutral buffered 10% formalin (except the testis and epididymis, which were fixed in modified Davidson's solution). Additionally, the (XX) tissues were weighed (paired organs were weighed together).

	Males		Females		Both sexes
XX	Testis[a]	XX	Ovaries[d]	XX	Liver
XX	Epididymis[a, b]	XX	Oviducts[d]	XX	Kidneys
XX	Prostate	XX	Uterus[e]	XX	Spleen
XX	Seminal vesicles[c]	XX	Cervix[e]	XX	Brain
XX	Coagulating glands[c]	X	Vagina	XX	Pituitary gland[f]
				XX	Adrenal glands
				XX	Thyroid gland[f]
				X	Gross lesions

a The right testis and epididymis were fixed, while the left testis and epididymis were frozen for sperm evaluations.
b The right cauda epididymis was also weighed separately.
c The seminal vesicles and coagulating glands were weighed together.
d The ovaries and oviducts were weighed together.
e The uterus and cervix were weighed together.
f Weighed after fixation

All tissues from the controls and high dose groups and all mated P1 and F1 adults that failed to produce a litter were processed routinely, stained with hematoxylin and eosin, and examined microscopically. The thyroid of the F1 females in the intermediate dose groups were also examined. Microscopic examination of all tissues was performed on one 5000 ppm P1 female that was killed prematurely due to dystocia; microscopic examination of other early deaths in the intermediate dose groups was limited to examination of gross lesions. The numbers of primordial and growing follicles (up to but not including antral follicles) were determined in 10 lactating F1 females in the control and high dose groups. Six 5 µm cross sections from the central area of each ovary were cut using a step section technique, and enumerated in up to 12 sections per animal. The severity of the microscopic findings was graded as minimal, mild, moderate, or severe.

b. **Offspring:** Pups that died during the lactation period were necropsied and discarded; pups that were orphaned by the death of their dam were euthanized (method not provided), examined grossly, and discarded. Pups that were culled on PND 4 were euthanized by decapitation and discarded. F1 pups not selected on PND 21 as the F1 parents and all F2 pups were euthanized by exsanguination under carbon dioxide anesthesia and necropsied on PND 21. The brain, spleen, thymus, and thyroid gland were collected from randomly selected F1 and F2 weanlings (one pup/sex/litter); the brain, spleen, and thymus were also weighed. Gross lesions were collected from all weanlings. Tissues were preserved in formalin. The thyroid gland was processed and examined in the control and high dose groups.

D. DATA ANALYSIS

1. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure		
	Preliminary tests	Preliminary test is not significant	Preliminary test is significant
Body weight and weight gain, food consumption and efficiency, precoital interval, gestation length, implantation sites, post-implantation loss, number of pups/litter, live born, viability, and lactation indices, sperm parameters, estrus cycle length, organ weights, and ovarian follicle counts	Levine's test for homogeneity and Shapiro-Wilk test for normality[a]	One-way analysis of variance and Dunnett's test	Kruskal-Wallis test and Dunn's test
Mating and fertility indices	None	Sequential application of Cochran-Armitage test for trend[b]	
Sex ratio (covariate: litter size) Pup weights (covariates: litter size, sex ratio) Developmental landmarks (covariate: body weight)	Levine's test for homogeneity and Shapiro-Wilk test for normality[c]	Analysis of covariance and Dunnett-Hsu	Non-parametric analysis of covariance

a If the Shapiro-Wilk test was not significant but Levine's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was used, followed by Dunn's test if necessary.
b If the incidence was not significant, but a significant lack of fit occurred, then Fisher's Exact Test with a Bonferroni correction was used.
c A normalizing, variance stabilizing transformation may have been used as needed.

For litter parameters, the proportion of affected pups/litter or the litter mean was used as the experimental unit for statistical evaluation. Statistical significance was denoted at $p \leq 0.05$. The statistical methods were considered appropriate.

2. **Indices**

Reproductive indices: The following reproductive indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Mating index (%) = # of animals mated/# of animals paired x 100

Fertility index (%) = # of pregnant females/# of females mated x 100

Post-implantation loss (%) = (# of implantation sites - # of pups born)/# of implantation sites x 100

Offspring viability indices: The following offspring indices were calculated by the performing laboratory from lactation records of litters in the study:

Live birth index (%) = # of pups born alive/# pups born x 100

Viability index (%) = # of live pups on PND 4 (pre-cull)/# of pups born alive x 100

Lactation index (%) = # of live pups at weaning/# of live pups on PND 4 (post-cull) x 100

3. **Historical control data:** Historical control data were not provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** There were no treatment-related mortalities in either generation. All P males survived to scheduled termination. Three P females died prior to their scheduled euthanasia. One 17,000 ppm female (#554) was found dead on GD 19. This dam was observed with increased breathing, dehydration, and lack of feces present on the cage board; the cause of death was determined to be pulmonary arterial thrombosis. One 5000 ppm female (#452) was found dead on Day 4 due to pyelonephritis; this animal was replaced with female #478 on the following day. Another 5000 ppm female (#455) was killed *in extremis* on Day 99 after being observed with signs indicative of dystocia. These deaths were not considered to be a result of treatment. There were no other deaths in the P females.

In the F1 generation, one 5000 ppm male (#4611) was accidentally killed on Day 0 and replaced with male #4753 on the same day. One 500 ppm male (#2501) was found dead on Day 54; the cause of death was chronic progressive nephropathy. One 5000 ppm female (#4662) and one control female (#1582) were killed *in extremis* on Days 104 and 106, respectively, after being observed with signs indicative of dystocia. However, no conclusive pathological evidence of dystocia was observed in the 5000 ppm dam. There were no other deaths in the F1 parents.

b. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed. The most common observation was hair loss, observed in all groups in both males and females of both generations.

2. Body weight, body weight gain, food consumption, and food efficiency

a. **Pre-mating:** During the pre-mating period, treatment-related effects were observed on body weights, body weight gains, and food efficiency at 5000 ppm and above (Table 3a). In the P males, body weights were decreased ($p \leq 0.05$) by 6-7% during Days 42-105 at 5000 ppm and by 6-9% during Days 35-119 at 17,000 ppm. Pre-mating (Days 0-70) body weight gains were decreased ($p \leq 0.05$) by 14-17% at 5000 ppm and above, and overall (Days 0-119) body weight gains were decreased ($p \leq 0.05$, except not significant [NS] at 5000 ppm) by 10-13% at 5000 ppm and above. Food consumption was unaffected by treatment, but pre-mating food efficiency was decreased ($p \leq 0.05$) by 10-15% at 5000 ppm and above as a result of the decreased body weight gains. Pre-mating food efficiency was also decreased ($p \leq 0.05$) by 8% at 1500 ppm, but this finding was considered to be due to a transient decrease in body weight gains for Days 56-63. In the 17,000 ppm P females, body weights were decreased ($p \leq 0.05$) by 6-7% during Days 56-70, resulting in a 16% decrease ($p \leq 0.05$) in pre-mating

body weight gains. Pre-mating food efficiency was decreased ($p \leq 0.05$) by 12% due to the decreased body weight gains for this period.

In the F1 males, body weights were decreased ($p \leq 0.05$) by 7-9% during Days 63-105 at 5000 ppm and by 12-15% throughout treatment (Days 0-105) at 17,000 ppm. Pre-mating body weight gains were decreased ($p \leq 0.05$) by 7-13% at 5000 ppm and above, and overall (Days 0-105) body weight gains were decreased ($p \leq 0.05$) by 9-15% at 5000 ppm and above. Additionally at 17,000 ppm, pre-mating food consumption and food efficiency were decreased ($p \leq 0.05$) by 5% and 9%, respectively. In the 17,000 ppm F1 females, body weights were decreased ($p \leq 0.05$) by 10-14% during Days 0-14, but were similar to controls for the remainder of pre-mating; pre-mating body weight gains were also similar to controls. Food consumption and efficiency were unaffected by treatment during pre-mating.

TABLE 3a. Selected mean (±SD) body weights (g), body weight gains (g),food consumption (g/rat/day), and food efficiency during pre-mating[a]

Observation/study day [b]		Dose Group (ppm)				
		0	500	1500	5000	17,000
P Males (n=28)						
Body weight	Day 0	278.6±12.0	277.5±12.6	277.5±13.2	276.4±14.5	276.6±12.9
	Day 35	467.1±25.1	460.0±40.3	455.7±38.2	444.3±35.7	440.9±37.0* (↓6)
	Day 42	488.0±26.0	480.6±41.5	473.8±39.4	459.3±39.8* (↓6)	457.2±36.8* (↓6)
	Day 70	548.4±36.3	535.3±49.3	527.5±47.6	509.3±44.2* (↓7)	501.7±44.6* (↓9)
	Day 105	596.6±48.4	584.4±54.5	580.6±56.3	557.5±52.5* (↓7)	550.8±49.5* (↓8)
	Day 119	599.6±48.5	595.2±56.3	593.1±58.5	564.8±57.1	555.8±50.6* (↓7)
Body weight gain	Days 0-70	269.8±32.9	257.8±43.3	249.9±39.1	233.0±36.2* (↓14)	225.1±40.4* (↓17)
	Days 0-119	321.0±46.4	317.8±51.0	315.6±50.9	288.4±48.7 (↓10)	279.2±47.0* (↓13)
Food consumption	Days 0-70	26.8±1.8	26.8±2.8	27.1±2.2	25.8±2.2	26.4±2.3
Food efficiency	Days 0-70	0.143±0.013	0.137±0.015	0.131±0.012* (↓8)	0.129±0.012* (↓10)	0.121±0.014* (↓15)
P Females (n=30)						
Body weight	Day 0	194.0±11.2	193.0±10.7	193.0±10.4	191.6±10.7	191.6±11.9
	Day 56	293.4±23.4	289.0±24.6	295.2±25.4	283.6±21.7	273.6±19.3* (↓7)
	Day 70	300.9±24.1	296.5±26.0	303.2±25.7	294.9±22.9	281.6±19.3* (↓6)
Body weight gain	Days 0-70	106.8±19.2	103.4±22.1	110.2±21.9	102.5±18.3	90.0±17.0* (↓16)
Food consumption	Days 0-70	19.6±2.3	18.9±1.5	19.7±1.8	19.1±1.8	18.6±1.2
Food efficiency	Days 0-70	0.078±0.012	0.078±0.012	0.080±0.010	0.076±0.009	0.069±0.011* (↓12)
F1 Males (n=22-24)						
Body weight	Day 0	59.3±6.7	55.4±5.0	56.0±5.7	55.9±5.6	50.5±5.4* (↓15)
	Day 21	234.8±21.0	231.8±20.5	231.0±22.5	226.9±18.3	207.0±14.4* (↓12)
	Day 63	513.2±49.1	499.0±41.3	500.0±58.2	477.9±47.1* (↓7)	447.8±32.6* (↓13)
	Day 105	615.8±57.8	588.3±56.6	591.1±77.7	561.1±62.5* (↓9)	521.2±49.9* (↓15)
Body weight gain	Days 0-70	478.0±50.8	460.9±45.1	467.9±60.3	444.2±49.4* (↓7)	416.3±33.1* (↓13)
	Days 0-105	556.5±56.8	532.8±55.0	535.0±75.2	505.5±60.0* (↓9)	470.8±49.6* (↓15)
Food consumption	Days 0-70	26.5±3.3	26.3±1.9	26.3±2.9	25.7±2.7	25.1±1.3* (↓5)
Food efficiency	Days 0-70	0.261±0.042	0.250±0.011	0.253±0.011	0.247±0.014	0.237±0.012* (↓9)
F1 Females (n=22-24)						
Body weight	Day 0	55.1±6.8	53.4±6.5	53.8±5.8	52.1±5.5	47.5±4.7* (↓14)
	Day 14	137.1±13.8	137.3±13.5	139.3±12.5	135.9±10.5	123.6±9.3* (↓10)
	Day 70	284.9±38.7	293.4±31.3	286.7±34.2	280.3±25.8	266.1±22.4
Body weight gain	Day 0-70	229.9±37.1	240.1±31.4	232.9±32.9	228.2±23.6	218.6±21.4
Food consumption	Days 0-70	18.8±2.1	18.9±1.7	19.1±2.0	18.7±1.9	18.4±1.5
Food efficiency	Days 0-70	0.174±0.015	0.181±0.011	0.173±0.010	0.175±0.011	0.170±0.090

a Data were obtained from Tables 17-20, 25-28, 33-36, and 41-44 on pages 82-87, 92-97, 102-105, and 110-113 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

* Significantly different from controls; p≤0.05

b. Gestation: In the P generation, body weights were decreased (p≤0.05) in the 17,000 ppm dams by 8% on GD 7-21, resulting in decreased (p≤0.05) gestational (GD 0-21) body weight gains of 12% (Table 3b). Additionally at this dose, food consumption was decreased (p≤0.05) by 10% during GD 7-14, and food efficiency was decreased (p≤0.05) by 20% during GD 0-7; however, food consumption and efficiency for the gestation period (GD 0-21) were similar to controls. There were no effects of treatment during gestation on body weights, body weight gains, or food consumption or efficiency in the 5000 ppm and below P dams or in the F1 generation.

TABLE 3b. Mean (±SD) body weights (g), body weight gains (g), food consumption (g/rat/day), and food efficiency during gestation[a]

Observation/gestation day		Dose Group (ppm)				
		0	500	1500	5000	17,000
P Females						
Body weight	GD 0	300.3±27.4	298.4±28.1	304.0±28.2	293.9±23.3	283.9±17.3
	GD 7	342.5±28.1	335.2±28.7	345.6±31.5	331.7±23.3	315.4±23.5* (↓8)
	GD 14	373.0±27.4	362.7±29.7	377.8±31.9	358.0±24.7	342.9±23.9* (↓8)
	GD 21	457.5±35.6	442.0±40.0	463.5±36.6	443.0±28.7	422.0±30.0* (↓8)
Body weight gain	GD 0-21	157.2±20.3	143.6±24.3	158.5±22.7	149.1±23.4	138.0±21.3* (↓12)
Food consumption	GD 7-14	26.9±2.3	25.5±2.7	26.6±3.2	25.4±3.6	24.3±2.3*(↓10)
Food efficiency	GD 0-7	0.245±0.053	0.229±0.036	0.238±0.049	0.234±0.070	0.195±0.051*(↓20)
F1 Females						
Body weight	GD 0	297.0±45.4	303.4±35.1	292.3±30.2	291.9±28.4	273.7±25.5 (↓8)
	GD 7	331.1±44.6	330.6±46.6	330.2±35.7	326.3±29.9	306.3±28.1 (↓7)
	GD 14	362.2±46.5	366.5±37.0	362.7±35.7	359.6±31.7	338.3±29.4 (↓7)
	GD 21	442.6±56.5	449.7±41.4	445.3±42.7	445.4±46.1	423.0±34.8 (↓4)
Body weight gain	GD 0-21	145.5±27.2	143.1±16.4	153.1±21.4	153.8±30.4	148.8±14.7

a Data were obtained from Tables 21-22 and 29-30 on pages 88-89 and 98-99 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

* Significantly different from controls: p≤0.05

c. Lactation: In the P generation, body weights were decreased (p≤0.05) in the 17,000 ppm dams by 7-9% on LD 0-7; body weights were similar to controls for the remainder of lactation (Table 3c). Lactational (LD 0-21) body weight gains were unaffected by treatment. Additionally at this dose, food consumption was increased (p≤0.05) by 8% during LD 7-14; food efficiency was unaffected by treatment. There were no effects of treatment during lactation on body weights, body weight gains, or food consumption or efficiency in the 5000 ppm and below P dams or in the F1 generation.

TABLE 3c. Mean (±SD) body weights (g) and body weight gains (g) during lactation[a]						
Observation/lactation day		Dose Group (ppm) 0	500	1500	5000	17,000
P Females						
Body weight	LD 0	349.8±34.3	342.4±29.6	349.6±32.9	334.9±26.6	319.1±26.4* (↓9)
	LD 7	359.3±27.8	351.7±27.3	359.6±24.5	349.1±23.4	335.6±17.5* (↓7)
	LD 14	355.8±30.3	352.0±25.9	356.8±31.1	343.7±29.2	339.0±24.1
	LD 21	350.3±24.8	344.0±23.1	347.7±20.8	334.9±26.0	333.8±19.2
Body weight gain	LD 0-21	0.5±15.3	3.5±16.2	-1.9±22.2	0.0±17.7	13.2±24.5
F1 Females						
Body weight	LD 0	338.2±50.7	340.7±40.2	339.1±33.3	332.0±28.0	315.5±26.7 (↓7)
	LD 7	353.3±40.5	354.5±26.1	345.6±33.4	351.7±26.4	332.1±33.7 (↓6)
	LD 14	359.3±40.7	364.3±27.1	358.5±27.2	363.4±24.6	347.7±32.7 (↓3)
	LD 21	350.1±40.9	346.5±25.7	341.8±25.2	340.7±21.3	337.9±28.9 (↓3)
Body weight gain	LD 0-21	17.7±29.1	5.8±24.4	2.9±19.0	8.7±20.8	21.9±14.6

a Data were obtained from Tables 23-24 and 31-32 on pages 90-91 and 100-101 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.

* Significantly different from controls; p≤0.05

3. **Test substance intake:** Test substance intakes (mg/kg/day) for pre-mating, gestation, and lactation are presented in Table 4.

TABLE 4. Mean (±SD) test compound intake (mg/kg/day) during pre-mating, gestation, and lactation[a]						
Group		Dose Group (ppm) 0	500	1500	5000	17,000
Pre-mating						
P generation males	Days 0-70	0.0±0.0	30.11±1.41	91.93±2.85	299.09±11.28	1048.26±49.63
F1 generation males	Days 0-70	0.0±0.0	42.32±1.56	126.33±4.11	425.76±16.93	1521.77±68.08
Males average mean intake[b]		0.0	36.22	109.13	362.42	1285.02
P generation females	Days 0-70	0.0±0.0	35.97±2.00	110.02±5.76	367.00±21.63	1243.26±58.13
F1 generation females	Days 0-70	0.0±0.0	46.42±2.38	140.93±6.36	465.20±17.33	1665.55±65.86
Females average mean intake[b]		0.0	41.20	125.48	416.10	1454.41
Gestation						
P females	GD 0-21	0.0±0.0	33.03±2.37	100.00±5.75	330.51±23.13	1158.02±56.53
F1 females	GD 0-21	0.0±0.0	32.22±2.32	101.88±5.92	335.75±16.58	1192.19±65.75
Lactation						
P females	LD 0-14	0.0±0.0	58.53±8.12	174.72±16.08	599.47±69.38	2214.79±250.65
F1 females	LD 0-14	0.0±0.0	64.17±5.21	189.05±23.20	651.05±61.88	2243.44±820.82

a Data were obtained from Tables 1-8 on pages 58-65 of the study report.

b Calculated by reviewers from data presented in this table.

4. **Reproductive function**

a. **Estrus cycle length and periodicity:** There were no effects on estrus cyclicity in either the P or F1 dams. At 5000 ppm, the mean cycle length was increased (p≤0.05) in the F1 females (4.7 days treated vs. 4.1 days controls). However, this was primarily due to an unusually high value observed for one dam (#4652). Since the mean cycle length was unaffected by treatment at 17,000 ppm in the F1 generation, and there were no effects observed in the P generation, this finding was considered incidental.

b. **Sperm measures:** No treatment-related differences were observed in any of the measured sperm parameters in either generation.

5. **Reproductive performance:** Reproductive performance data are presented in Table 5. There were no effects of treatment on mating, fertility, pre-coital interval length, gestation length, number of implantation sites, or post-implantation loss. Post-implantation loss was decreased ($p \leq 0.05$) in the 5000 ppm F1 dams (2.3 treated vs. 10.1 controls); however, decreases in this parameter are not normally considered adverse.

TABLE 5. Reproductive performance[a]

Observation/study week	Dose Group (ppm)				
	0	500	1500	5000	17,000
P generation					
Females paired	28	28	28	28	28
Females with evidence of copulation	28	28	27	28	26
Females pregnant	23	26	24	25	24
Females that littered	21	26	24	23	24
Mating index (%)[b] (calculated by reviewer)	100	100	100 (96.4)	100	96.4 (92.8)
Fertility index (%)[c] (calculated by reviewer)	82.1	92.9	85.7 (88.9)	89.3	88,9 (92.3)
Pre-coital interval (days)	2.3±1.5	2.6±1.9	2.5±1.8	2.7±1.7	3.0±2.3
Gestation length (days)	22.3±0.5	22.3±0.7	22.2±0.5	22.2±0.4	22.3±1.1
Number of implantation sites	13.4±4.6	13.2±4.1	15.0±1.7	13.8±4.1	14.3±2.8
Post-implantation loss (%)[d]	3.6±6.5	2.9±5.6	4.4±6.8	3.6±8.2	5.4±8.4
F1 generation					
Females paired	28	28	28	28	28
Females with evidence of copulation	26	22	28	26	25
Females pregnant	24	19	25	25	25
Females that littered	23	19	25	24	25
Mating index (%)[b] (calculated by reviewer)	92.9	78.6	100	96.4 (92.9)	96.4 (89.3)
Fertility index (%)[c] (calculated by reviewer)	92.3	86.4	89.3	92.6 (96.1)	92.6 100
Pre-coital interval (days)	3.1±2.3	3.6±3.4	3.1±2.6	3.5±1.8	3.8±3.4
Gestation length (days)	22.2±0.7	22.0±0.4	22.2±0.5	22.0±0.3	22.0±0.4
Number of implantation sites	14.3±4.3	14.9±1.8	14.8±2.5	15.0±2.8	15.4±2.0
Post-implantation loss (%)[d]	10.1±10.0	7.7±10.2	5.5±7.3	2.3±3.8*	4.1±6.4

a Data were obtained from Tables 53-54 on pages 122-123 of the study report.
b Mating index (%) = # of animals mated/# of animals paired x 100 (values given in study report)
c Fertility index (%) = # of pregnant females/# of females mated x 100 (values given in study report)
d Post-implantation loss (%) = (# of implantation sites - # of pups born)/# of implantation sites x 100
* Significantly different from controls; $p \leq 0.05$

There were no effects of treatment on reproductive failure in the parents. The incidence of reproductive failure in the P adults was 5/28, 2/28, 4/28, 3/28, and 4/28 in the 0, 500, 1500, 5000, and 17,000 ppm groups, respectively, the incidence of reproductive failure in the F1 adults was 4/28, 9/28, 3/28, 3/28, and 3/28 in the 0, 500, 1500, 5000, and 17,000 ppm groups, respectively.

Parental postmortem results

a. **Organ weights:** There were no effects of treatment on organ weights in either generation. The 1500 ppm and above P males had increased ($p \leq 0.05$; except NS at 1500 ppm) absolute and relative (to body) adrenal weights (↑11-14%; Table 6a). However, there was little increase in weight with dose, and no corroborating microscopic findings. Therefore, this finding was considered incidental. Terminal body weights were decreased ($p \leq 0.05$) by 7% in the 17,000 ppm males, resulting in similar increases ($p \leq 0.05$) in the relative weights of the epididymides (↑9%), kidneys (↑5%), right cauda epididymis (↑10%), seminal vesicles (↑18%), and testes (↑10%); absolute weights of these organs were similar to controls. Additionally, there were no corroborating microscopic findings in these organs. In the P females, slight decreases ($p \leq 0.05$) in absolute brain weight were noted at 5000 ppm and above (↓4%). As terminal body weights were similarly decreased ($p \leq 0.05$; except NS at 5000 ppm) by 4-6%, and no corroborating microscopic finding were observed, the brain weight decreases were considered incidental. There were no other changes observed in parental organ weights.

TABLE 6a. Selected mean (±SD) absolute (g) and relative to body (%) organ weights in the P generation[a]

Observation/study week		Dose Group (ppm)				
		0	500	1500	5000	17,000
Males						
Terminal body weight		602.5±51.2	596.8±55.1	598.0±60.1	573.7±58.3	562.3±52.2* (↓7)
Adrenal glands	absolute	0.051±0.007	0.054±0.008	0.057±0.010 (↑12)	0.057±0.010* (↑12)	0.058±0.008* (↑14)
	relative	0.009±0.001	0.009±0.001	0.010±0.002	0.010±0.002* (↑11)	0.010±0.001* (↑11)
Epididymides	absolute	1.667±0.158	1.574±0.203	1.683±0.162	1.645±0.157	1.694±0.151
	relative	0.280±0.032	0.266±0.044	0.283±0.026	0.289±0.034	0.304±0.040* (↑9)
Kidneys	absolute	4.053±0.620	3.944±0.344	4.058±0.388	3.943±0.428	3.990±0.458
	relative	0.674±0.100	0.662±0.039	0.680±0.048	0.690±0.065	0.711±0.067* (↑5)
Right cauda epididymis	absolute	0.352±0.049	0.332±0.046	0.357±0.037	0.349±0.036	0.365±0.030
	relative	0.059±0.009	0.056±0.010	0.060±0.006	0.061±0.008	0.065±0.008* (↑10)
Seminal vesicles	absolute	2.453±0.417	2.423±0.602	2.532±0.490	2.636±0.366	2.689±0.412
	relative	0.409±0.071	0.409±0.107	0.427±0.087	0.462±0.063	0.483±0.091* (↑18)
Testes	absolute	3.633±0.321	3.596±0.604	3.706±0.312	3.723±0.317	3.746±0.331
	relative	0.607±0.074	0.607±0.115	0.624±0.066	0.655±0.085	0.670±0.077* (↑10)
Females						
Terminal body weight		348.2±25.5	342.7±22.5	345.4±21.2	332.8±26.8 (↓4)	328.2±22.4* (↓6)
Brain	absolute	2.031±0.092	1.999±0.088	1.996±0.075	1.958±0.125* (↓4)	1.940±0.129* (↓4)
	relative	0.586±0.044	0.586±0.044	0.580±0.040	0.591±0.048	0.593±0.051

a Data were obtained from Tables 63-64 on pages 134-143 of the study report.
* *Significantly different from controls;* $p \leq 0.05$

In the F1 males, terminal body weights were decreased ($p \leq 0.05$) by 9-16% at 5000 ppm and above, resulting in decreases ($p \leq 0.05$) in the absolute weights of the testes (↓5%), and increases ($p \leq 0.05$) in the relative weight of the adrenals (↑11%), brain (↑7-14%), and seminal vesicles (↑12-22%; Table 6b). Additionally at 17,000 ppm, decreases ($p \leq 0.05$) in absolute brain (↓4%), liver (↓17%), spleen (↓16%), and thyroid (↓17%), and increases ($p \leq 0.05$) in epididymides (↑15%), pituitary (↑50%), right cauda epididymis (↑16%), and

testis (↑12%) were observed. As there were no corroborating microscopic findings in these organs, these weight changes were all attributed to the decreased terminal body weights. In the F1 females, slight decreases ($p \leq 0.05$) in absolute brain weights were noted at 17 ,000 ppm (↓5%). As terminal body weights were similarly decreased (NS) by 4%, and no corroborating microscopic finding were observed, the brain weight decreases were considered incidental. Absolute testes weights were also decreased ($p \leq 0.05$) by 6% in the 500 ppm males, but this finding was not dose-dependent and was considered incidental.

TABLE 6b. Selected mean (±SD) absolute (g) and relative to body (%) organ weights in the F1 generation[a]

Observation/study week		Dose Group (ppm)				
		0	500	1500	5000	17,000
Males						
Terminal body weight		644.1±60.5	616.1±60.1	620.4±81.3	587.3±65.2* (↓9)	543.2±49.5* (↓16)
Adrenal glands	absolute	0.056±0.007	0.055±0.012	0.056±0.009	0.059±0.011	0.057±0.008
	relative	0.009±0.001	0.009±0.002	0.009±0.002	0.010±0.002* (↑11)	0.010±0.002* (↑11)
Brain	absolute	2.259±0.105	2.216±0.128	2.198±0.097	2.193±0.091	2.173±0.090* (↓4)
	relative	0.353±0.030	0.362±0.036	0.359±0.043	0.378±0.042* (↑7)	0.403±0.033* (↑14)
Epididymides	absolute	1.688±0.225	1.713±0.178	1.673±0.230	1.611±0.146	1.637±0.151
	relative	0.264±0.038	0.279±0.028	0.271±0.032	0.277±0.031	0.303±0.031* (↑15)
Liver	absolute	21.948±2.805	21.068±2.543	21.672±3.986	20.103±3.275	18.263±2.260* (↓17)
	relative	3.403±0.231	3.418±0.221	3.478±0.262	3.409±0.273	3.357±0.198
Pituitary	absolute	0.015±0.002	0.014±0.002	0.015±0.002	0.014±0.003	0.014±0.003
	relative	0.002±0.000	0.002±0.000	0.002±0.000	0.002±0.000	0.003±0.000* (↑50)
Right cauda epididymis	absolute	0.368±0.049	0.365±0.038	0.360±0.042	0.355±0.040	0.355±0.036
	relative	0.057±0.008	0.060±0.008	0.059±0.007	0.061±0.009	0.066±0.007* (↑16)
Seminal vesicles	absolute	2.279±0.327	2.363±0.426	2.240±0.360	2.336±0.316	2.360±0.407
	relative	0.357±0.059	0.385±0.068	0.365±0.063	0.400±0.052* (↑12)	0.434±0.061* (↑22)
Spleen	absolute	0.979±0.150	0.908±0.090	0.957±0.155	0.900±0.135	0.825±0.139* (↓16)
	relative	0.152±0.020	0.148±0.013	0.155±0.023	0.153±0.017	0.152±0.021
Testes	absolute	3.989±0.338	3.757±0.342* (↓6)	3.816±0.329	3.774±0.263* (↓5)	3.771±0.275* (↓5)
	relative	0.622±0.058	0.614±0.066	0.622±0.071	0.650±0.080	0.698±0.064* (↑12)
Thyroid	absolute	0.030±0.008	0.029±0.007	0.029±0.006	0.029±0.007	0.025±0.005* (↓17)
	relative	0.005±0.001	0.005±0.001	0.005±0.001	0.005±0.001	0.005±0.001
Females						
Terminal body weight		349.1±42.4	345.6±28.1	345.6±30.9	337.9±22.3	335.5±28.6 (↓4)
Brain	absolute	2.042±0.105	2.042±0.112	2.003±0.089	2.034±0.112	1.940±0.079* (↓5)
	relative	0.601±0.083	0.594±0.048	0.583±0.048	0.604±0.042	0.583±0.058

a Data were obtained from Tables 65-66 on pages 144-151 of the study report.
* Significantly different from controls; $p \leq 0.05$

b. Pathology

1) **Macroscopic examination:** There were no treatment-related gross pathological findings in either generation.

2) **Microscopic examination:** There were no treatment-related microscopic findings. The numbers of primordial and pre-antral follicles were similar between the control and 17,000 ppm F1 females. Minimal thyroid follicular cell hypertrophy, characterized by an increase

in the size of the follicular lining cell and a change in shape from flat or cuboidal cell to columnar, was observed in 8/28 17,000 ppm F1 females compared to 2/28 controls. This finding was considered to be treatment-related, but due to the minimal severity was not considered adverse.

B. OFFSPRING

1. **Viability and clinical signs:** Litter parameters are presented in Table 7. There were no effects of treatment on either the F1 or F2 offspring from birth throughout lactation. Survival indices as well as means for litter sizes were comparable across all groups tested. Sex ratio (% males) was increased ($p \leq 0.05$) in the 1500 ppm F1 pups (56.7% treated vs. 44.9% controls) and decreased ($p \leq 0.05$) in the 1500 ppm F2 pups (43.8% treated vs. 50.6% controls). As dose-dependency was not observed, these findings were considered incidental.

   There were no test substance-related clinical observations in either the F1 or F2 offspring. The most commonly observed finding was eye closed in the F2 pups; this finding occurred in a non-dose-dependent fashion.

TABLE 7. Litter parameters[a]

Parameter	Dose Group (ppm)				
	0	500	1500	5000	17,000
F1 generation					
Number of viable litters:					
PND 0	21	25	24	23	23
PND 4	21	25	24	23	22
PND 7	21	25	24	23	22
PND14	21	25	24	23	22
PND 21	21	25	24	23	22
Number of pups:					
Born	13.9±3.7	13.0±4.0	14.4±2.0	13.7±3.6	13.6±3.1
Born alive	13.6±3.8	12.7±4.2	14.0±2.1	13.7±3.7	13.0±3.4
Number of pups alive:					
PND 0	13.6±3.8	12.7±4.2	14.0±2.1	13.7±3.7	13.0±3.4
PND 4 pre-cull	13.4±3.8	13.1±3.4	13.8±2.0	13.6±3.7	13.0±2.0
PND 4 post-cull	7.6±1.4	7.7±1.2	8.0±0.0	7.7±1.1	8.0±0.0
PND 7	7.5±1.4	7.7±1.2	8.0±0.2	7.7±1.1	8.0±0.0
PND 14	7.5±1.4	7.7±1.2	8.0±0.2	7.7±1.1	8.0±0.0
PND 21	7.5±1.4	7.7±1.2	7.9±0.3	7.7±1.1	7.8±1.1
Live birth index[b]	97.4±5.6	94.3±19.5	97.1±5.0	99.2±2.7	92.8±20.4
Viability index[c]	98.9±2.8	99.4±2.0	98.3±3.5	99.7±1.5	94.9±10.8
Lactation index[d]	98.9±3.6	99.5±2.4	98.5±4.1	100±0.0	97.2±13.2
Sex ratio	44.9±11.6	50.8±19.0	56.7±14.8*	49.4±13.8	50.0±19.7
F2 generation					
Number of viable litters:					
PND 0	22	19	25	24	25
PND 4	21	19	25	24	25
PND 7	21	19	25	24	25
PND14	21	19	25	24	25
PND 21	21	19	25	24	25
Number of pups:					
Born	13.1±4.1	13.7±2.2	13.9±2.5	14.8±2.9	14.7±1.9
Born alive	12.8±4.1	13.6±2.2	13.8±2.5	14.7±2.8	14.4±2.1
Number of pups alive:					
PND 0	12.8±4.1	13.6±2.2	13.8±2.5	14.7±2.8	14.4±2.1
PND 4 pre-cull	13.1±2.9	13.6±2.2	13.6±2.5	14.6±2.8	14.3±2.2
PND 4 post-cull	7.9±0.5	8.0±0.0	8.0±0.2	7.9±0.4	8.0±0.0
PND 7	7.8±0.6	8.0±0.0	8.0±0.2	7.9±0.4	8.0±0.2
PND 14	7.8±0.6	8.0±0.0	7.9±0.3	7.9±0.4	8.0±0.2
PND 21	7.8±0.6	7.9±0.3	7.9±0.3	7.8±0.5	8.0±0.2
Live birth index[b]	93.8±21.4	99.3±2.2	99.5±2.6	99.0±3.9	98.2±6.4
Viability index[c]	98.9±3.7	99.6±1.6	98.5±3.1	99.8±1.2	98.8±3.5
Lactation index[d]	98.8±5.5	98.7±3.8	99.5±2.4	99.0±3.4	99.5±2.4
Sex ratio	50.6±16.4	47.9±14.1	43.8±12.9*	45.6±11.0	50.4±10.8

a Data were obtained from Tables 55-56 on pages 124-125 of the study report.
b Live birth index (%) = # of pups born alive/# pups born x 100 (values given in study report)
c Viability index (%) = # of live pups on PND 4 (pre-cull)/# of pups born alive x 100 (values given in study report)
d Lactation index (%) = # of live pups at weaning/# of live pups on PND 4 (post-cull) x 100 (values given in study report)
* Significantly different from controls; $p \leq 0.05$

2. **Body weight:** Pup body weight and body weight gain data are presented in Table 8. In the 17,000 ppm F1 pups, body weights were decreased ($p \leq 0.05$; except NS on PND 7) by 7-15% from PND 0 through PND 21, with body weight gains decreased by 26% during PND 14-21. Overall pup weight gains (PND 0-21) were decreased by 16% (calculated by reviewers). Body weights were also decreased ($p \leq 0.05$) in the 5000 ppm F1 pups and the 17,000 ppm F2 pups on PND 21 by 7% and 8%, respectively, resulting in overall (PND 0-21) pup weight gain decreases of 8%. These decreases were not considered adverse due to the low magnitude and transient nature of the change. There were no other treatment-related effects on body weights in the pups of either generation.

TABLE 8. Mean (±SD) pup weights and weight gains (g)[a]

Post-natal day (PND)		Dose Group (ppm)				
		0	500	1500	5000	17,000
F1 pups						
Body weight	PND 0	7.1±0.7	6.8±0.8* (↓4)	6.7±0.5	7.0±0.6	6.6±0.5* (↓7)
	PND 4	11.2±1.9	11.0±2.3	10.8±1.1	10.7±1.8	10.4±0.9* (↓7)
	PND 7	17.9±2.8	17.6±3.0	17.4±1.6	17.1±2.1	16.7±1.3 (↓7)
	PND 14	35.9±4.9	34.6±3.7	34.9±2.6	34.5±2.7	33.0±1.8* (↓8)
	PND 21	58.5±6.5	56.3±5.7	56.3±4.7	54.5±5.0* (↓7)	49.6±4.3* (↓15)
Body weight gain	PND 0-4	4.1	4.2	4.1	3.7	3.8
	PND 4-7	6.7	6.6	6.6	6.4	6.3
	PND 7-14	18.0	17.0	17.5	17.4	16.3
	PND 14-21	22.6	21.7	21.4	20.0	16.6 (↓26)
	PND 0-21[b]	51.4	49.5	49.6	47.5 (↓8)	43.0 (↓16)
F2 pups						
Body weight	PND 0	6.7±0.7	6.5±0.4	6.6±0.7	6.4±0.5	6.5±0.4
	PND 4	11.1±1.5	10.8±0.8	11.0±1.4	10.3±1.2	10.4±0.8
	PND 7	17.8±2.1	17.6±1.5	17.5±2.0	16.9±1.8	17.0±1.1
	PND 14	37.3±3.1	37.1±2.5	36.5±3.1	36.5±2.7	35.6±2.5
	PND 21	59.5±4.6	59.1±4.1	59.4±5.1	57.1±5.6	55.0±4.0* (↓8)
Body weight gain	PND 0-4	4.4	4.3	4.4	3.9	3.9
	PND 4-7	6.7	6.8	6.5	6.6	6.6
	PND 7-14	19.5	19.5	19.0	19.6	18.6
	PND 14-21	22.2	22.0	22.9	20.6	19.4
	PND 0-21[b]	52.8	52.6	52.8	50.7	48.5 (↓8)

a Data were obtained from Tables 59 a, b, and c and 60 a, b, and c on pages 128-131 of the study report.
b Calculated by reviewers from data presented in this table.
* Significantly different from controls; $p \leq 0.05$

3. **Developmental landmarks:** There were no treatment-related effects on achievement of preputial separation or vaginal patency in the F1 offspring.

5. **Offspring postmortem results**

a) **Organ weights:** There were no effects of treatment on offspring organ weights. At 17,000 ppm, absolute and relative (to body) spleen weights were decreased ($p \leq 0.05$) by 13-31% in the F1 weanlings, and by 7-16% (NS relative weight in males and absolute and relative

weight in females) in the F2 weanlings; however, terminal body weights were decreased ($p \leq 0.05$) by 9-15% in these animals. As there were no treatment-related effects on organ weights or microscopic findings in the spleen of the P and F1 adults, it was considered that the decreased offspring spleen weights were a result of retarded spleen growth.

TABLE 10. Mean (±SD) absolute (g) and relative to body (%) weanling spleen weights[a]

Post-natal day (PND)		Dose Group (ppm)				
		0	500	1500	5000	17,000
F1 Male pups						
Terminal body weight (g)		59.5±4.8	56.8±6.1	56.3±5.0	53.1±6.2* (↓11)	51.0±4.8* (↓14)
Spleen	absolute	0.266±0.040	0.254±0.047	0.238±0.046	0.232±0.056	0.199±0.036* (↓25)
	relative	0.448±0.051	0.448±0.058	0.064	0.433±0.071	0.391±0.060* (↓13)
F1 Female pups						
Terminal body weight (g)		56.0±5.0	54.8±6.1	55.0±5.8	52.2±5.8	47.7±3.8* (↓15)
Spleen	absolute	0.264±0.050	0.243±0.039	0.247±0.037	0.232±0.062	0.182±0.026* (↓31)
	relative	0.471±0.079	0.443±0.050	0.450±0.056	0.444±0.093	0.382±0.046* (↓19)
F2 Male pups						
Terminal body weight (g)		61.9±5.6	60.2±4.8	60.7±6.0	59.0±6.5	56.1±5.9* (↓9)
Spleen	absolute	.289±0.045	0.253±0.045	0.275±0.063	0.256±0.064	0.243±0.042* (↓16)
	relative	0.467±0.060	0.420±0.065	0.450±0.082	0.432±0.089	0.432±0.048 (↓7)
F2 Female pups						
Terminal body weight (g)		57.8±5.5	58.0±4.9	57.6±6.2	55.5±7.4	52.3±6.8* (↓10)
Spleen	absolute	0.267±0.053	0.258±0.042	0.272±0.066	0.261±0.074	0.226±0.056 (↓15)
	relative	0.459±0.069	0.443±0.047	0.469±0.089	0.469±0.133	0.424±0.082 (↓8)

a Data were obtained from Tables 67-70 on pages 153, 155, 156, 158, 159, 161, 162, and 164 of the study report.
* Significantly different from controls; $p \leq 0.05$

b) Pathology

1) **Macroscopic examination:** There were no treatment-related macroscopic findings in either the F1 or F2 pups or weanlings.

2) **Microscopic examination:** There were no treatment-related microscopic findings in the F1 or F2 weanlings. Microscopic examinations were confined to the thyroid gland of the controls and 17,000 ppm F1 and F2 weanlings.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Under the experimental conditions of the current study, adverse test substance-related effects consisted of the following: systemic toxicity evident as effects on adult body weight parameters and nutritional parameters (food consumption and/or food efficiency) in P1 and F1 adult males at 5000 ppm and above and P1 and F1 adult females at 17,000 ppm. Treatment-related microscopic histopathological findings in adults consisted of minimal thyroid follicular cell hypertrophy in F1 adult females and decreased absolute and relative spleen weights in F1 and F2 male and female weanlings at 17,000 ppm.

Test substance-related effects on offspring consisted of reduced offspring weights at 17,000 ppm in F1 and F2 litters.

Non-adverse, test substance-related effects were limited to a slight reduction in mean offspring weight at 5000 ppm on lactation day 21. The magnitude of the reduction was small (5% and 6% lower than controls for males and females, respectively). This reduction is considered test substance related and is considered a reflection of the relatively high levels of test substance intake during this period of rapid growth when offspring are still nursing and also consuming the test diets; offspring weights were comparable to controls through lactation day 14 when the pups are nursing and unable to consume test diets directly. This reduction at 5000 ppm is not considered adverse because of the minimal magnitude and the transient nature of the reduction. On lactation day 21, selected pups are randomly assigned to serve as the parental generation for production of F2 litters. Thus, lactation day 21 is equal to test day 0 for the F1 generation adults. Despite the fact that the lactation day 21 offspring weights were lower, the test day 0 weights from a subset of these offspring on the same day were generally comparable to controls, as were the body weight gains for this group throughout premating for 5000 ppm F1 rats. This observation underscores the minimal magnitude of this change. Lastly, this slight effect was even less pronounced among F2 offspring; at 5000 ppm, F2offspring weights were comparable to controls throughout lactation.

Therefore, the no-observed-adverse-effect level (NOAEL) for reproductive toxicity was 5000 ppm (367.0-465.2 mg/kg bw for P1 and F1 females) due to reduced offspring weight at 17,000 ppm and the NOAEL for systemic toxicity was 1500 ppm (91.9-126.3 mglkg bw) for P1 and F1 males and 5000 ppm (367.0-465.2 mg/kg bw) for P1 and F1 females, based on reduced body weight and food parameters at 5000 and 17,000 ppm, respectively.

B. REVIEWER'S COMMENTS

1. **PARENTAL ANIMALS:** No treatment-related effects were observed on mortality, clinical signs, organ weights, or macroscopic or microscopic findings.

One 17,000 ppm P female (#554) was found dead on GD 19. This dam was observed with increased breathing, dehydration, and lack of feces present on the cage board; the cause of death was determined to be pulmonary arterial thrombosis. One 5000 ppm P female (#452) was found dead on Day 4 due to pyelonephritis; this animal was replaced with female #478 on the following day. Another 5000 ppm P female (#455) was killed *in extremis* on Day 99 after being observed with signs indicative of dystocia. One 5000 ppm F1 male (#4611) was accidentally killed on Day 0 and replaced with male #4753 on the same day. One 500 ppm F1 male (#2501) was found dead on Day 54; the cause of death was chronic progressive nephropathy. One 5000 ppm F1 female (#4662) and one control F1 female (#1582) were killed *in extremis* on Days 104 and 106, respectively, after being observed with signs indicative of dystocia. However, no conclusive pathological evidence of dystocia was observed in the 5000 ppm dam. These deaths were not considered to be a result of treatment.

Body weights and body weight gains were significantly ($p \leq 0.05$) decreased in P and F1 parental males over the pre-mating interval (Days 0-70) at 5000 ppm and above. Body weight gains were decreased (↓14%) in P males and (↓7%) in F1 males at 5000 ppm. Food efficiency was also decreased (↓10%) for P males only. At 17, 000 ppm, body weight gains were decreased (↓17%) in P males and (↓13%) in F1 males. Food efficiency was decreased (↓15%) and (↓9%), respectively. The only change in food consumption was for F1 males at 17,000 ppm (↓5%). Body weights and body weight gain were significantly ($p \leq 0.05$) decreased in P females over the pre-mating period (Days 0-70) at 17,000 ppm only. Body weight gain was decreased (↓16%) and food efficiency was decreased (↓12%). Food consumption was unchanged. For F1 females, body weights were significantly decreased through Day 14, but returned to control levels by Day 70. No changes in body weight changes were observed, and food consumption and food efficiency were unaffected.

Over the gestational period, body weight in P females was significantly ($p \leq 0.05$) decreased (↓8%) by GD 7 and body weight gain from GD 0-21 was decreased (↓12%) at 17,000 ppm. However body weights were not significantly different from controls by LD 14. Body weight gains for 17,000 ppm females were from LD 0-21 were 13.2 ± 24.5 g compared to 0.5 ± 15.3 g in controls. No differences in body weight or body weight gain were observed for F1 females during lactation.

Terminal body weights were significantly ($p \leq 0.05$) decreased at 17,000 ppm for P males (↓7%) and F1 males (↓16%). There were statistically significant changes in organ weights at 5,000 and 17,000 ppm for P and F1 animals, primarily in males. At 5,000 ppm, terminal body weight was also decreased (↓9%) for F1 males. There were statistically significant ($p \leq 0.05$) increases in absolute and/or relative (to terminal body weight) organ weights for P and F1 males at 5000 ppm (adrenal glands) and 17,000 ppm (and 17,000 ppm. However,

there were no microscopic findings in any of the organs. At 5,000 ppm, increased absolute and relative adrenal gland weights were observed for P males (↑11-12%). Relative adrenal weights were increased for F1 males (↑11%). Also at 5,000 ppm, relative seminal vesicle weight was increased (↑12%) and absolute weight of the testes was decreased (↓5%) for F1 males. At 17,000 ppm, absolute and relative adrenal weights were increased (↑11-14%) for P males and relative weights were increased (↑11%) for F1 males. Relative weights of the following organs for P, F1 males were changed at 17,000 ppm: epididymies (↑9%,↑15%), right cauda epididymis (↑10%,↑16%), seminal vesicles (↑18%, ↑22%), testes (↑10%, ↑12%). Other organ weight changes at 17,000 ppm for which there were no microscopic effects were decreased relative kidney weights in P males (↓5%) and increased relative pituitary weights in F1 males (↑50%). The terminal body weights were significantly ($p \leq 0.05$) decreased at 17,000 ppm for P females only (↓6%). The brain was the only organ for P and F1 females with significant changes in absolute weight at 5,000 ppm (↓4% for P females only) and 17,000 ppm (↓4% for P and ↓5% for F1). However, no changes in relative brain weights were observed for P or F1 females and no microscopic effects were found.

The reviewer did not consider the organ weight changes to be biologically significant, which contrasts from the investigator's conclusions. The rationale for this was that most of the changes were significant only after normalization to terminal body weight, which was reduced in higher dose animals, and there were no supporting microscopic changes. This reviewer agrees the changes in most relative organ weights were probably a consequence of decreased terminal body weight. This reviewer considers the finding of decreased brain weight at 5,000 and 17,000 ppm to be potentially adverse, even in the absence of microscopic effects. However, this finding has no impact on the NOAEL/LOAEL.

The LOAEL for parental toxicity is 17, 000 ppm (equivalent to 1285/1454 mg/kg in males/females), based on decreased body weights in parental males and females. The NOAEL is 5000 ppm (equivalent to 363/416 mg/kg in males/females).

2. **OFFSPRING:** No treatment-related effects on litter parameters were noted for the F1 or F2 generations. Specifically, there were no treatment-related effects were noted on the survival indices, clinical signs, sex ratio, attainment of developmental landmarks, or on gross or microscopic pathological findings.

In the 17,000 ppm F1 pups, body weights were significantly ($p \leq 0.05$) decreased (↓7-15%) from PND 0 through PND 21, with body weight gains decreased (↓26%) during PND 14-21. Overall pup body weight gains (PND 0-21) were decreased by (↓16%). Body weights were also decreased at PND 21 in the 5000 ppm F1 pups (↓7%) and the 17,000 ppm F2 pups (↓8%).

In weanlings, terminal body weights were significantly $p \leq 0.05$) decreased (↓11%) at 5,000 ppm in F1 males. At 17,000 pm, terminal body weights were significantly $p \leq 0.05$) decreased for all male and female F1 and F2 weanlings (↓9-15%). The following effects on absolute and relative spleen weights were also observed at 17,000 ppm. For F1 males and

females, absolute spleen weights were significantly decreased (↓25-31%) and relative (to body weight) spleen weights were decreased (↓13-19%). For F2 pups, only absolute spleen weights were significantly decreased for males (↓16%), although a similar, but non-significant decrease in absolute spleen weight (↓15%) was observed in females. Relative (to body weight) spleen weight decreases for F2 males and females were not statistically significant (↓7-8%).

This reviewer does not consider changes in absolute and relative spleen weights to be adverse because the open literature at the present time indicates that changes in spleen size/weight are not abnormal, and there were no microscopic effects.

The LOAEL for offspring toxicity is 17,000 ppm (equivalent to 1285/1454 mg/kg in males/females), based on decreased body weights and body weight gains in the F1 pups. The NOAEL is 5000 ppm (equivalent to 363/416 mg/kg in males/females).

3. **REPRODUCTIVE TOXICITY:** No treatment-related differences were observed in estrus cycle length or cyclicity, sperm parameters, numbers of primordial and pre-antral follicles, mating, fertility, pre-coital interval length, gestation length, number of implantation sites, or post-implantation loss, or reproductive failures.

The LOAEL for reproductive toxicity was not observed. The NOAEL is 17,000 ppm (equivalent to 1285/1454 mg/kg in males/females).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

D. STUDY DEFICIENCIES: No study deficiencies were observed.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR-METHYL (IN-KJM44)

Study Type: OPPTS 870.5100 [§84-2]; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 5-1-209 I (MRID 47560033)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/14/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Bacterial Gene Mutation *(Salmonella typhimurium)*/ mammalian activation gene mutation assay; OPPTS 870.5100 [' 84-2]; OECD 471.

PC CODES: 288009 **DP BARCODE:** D361256
TXR#: 0055188

TEST MATERIAL (PURITY): IN-KJM44 (aminocyclopyrachlor-methyl, 95.3% a.i.)

SYNONYMS: DPX-KJM44; Methyl 6-amino-5-chloro-2-(cyclopropyl)-4-pyrimidinecarboxylate

CITATION: Ford, L.S. (2005) IN-KJM44: Bacterial reverse mutation test. Haskell Laboratory for Health and Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-17971, November 18, 2005. MRID 47560033. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Newark, DE

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 47560033), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to IN-KJM44 (aminocyclopyrachlor-methyl, 95.3% a.i., Batch No.: 032) in DMSO at concentrations of 0, 100, 333, 1000, 3333, or 5000 µg/plate (+/-S9) using the standard plate incorporation method. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor® 1254. Standard strain-specific mutagens served as positive controls.

IN-KJM44 was tested up to the limit dose (5000 µg/plate). No evidence of cytotoxicity was observed at any concentration in any strain in either trial in the presence or absence of S9-activation. There were no marked increases in the mean number of revertants/plate in any strain. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. **There was no evidence of induced mutant colonies over background.**

The study is classified as **Acceptable/Non-guideline** and **does not satisfy** the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data. The study is upgradable to Acceptable, Guideline following receipt of GLP

compliance and Quality Assurance statements.

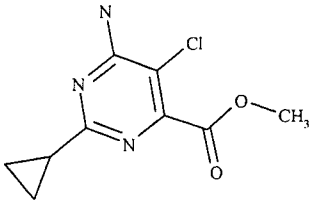
COMPLIANCE: A signed and dated Data Confidentiality statement was provided. The study was conducted in a GLP-compliant lab following SOPs but was not proven to be GLP compliant. A Quality Assurance statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-KJM44 (metabolite of Aminocyclopyrachlor)

Description: Beige solid
Batch #: 032
Purity: 95.3% a.i. (doses adjusted for purity)
Stability: Not reported
CAS # of TGAI: 858954-83-3 (Not reported in study report)
Structure:



2. Control materials

Negative: The solvent alone served as the negative control.
Solvent: Dimethylsulfoxide (DMSO; 100 µL/plate)

Positive:	Non-activation	Concentration	Strain
	Sodium azide	2.0 µg/plate	TA100, TA1535
	2-Nitrofluorene	1.0 µg/plate	TA98
	ICR-191	2.0 µg/plate	TA1537
	4-Nitroquinoline-N-oxide	1.0 µg/plate	WP2 *uvr*A
	Activation		
	2-Aminoanthracene	2.5 µg/plate	TA100, TA1535, and TA1537
		25 µg/plate	WP2 *uvr*A
	Benzo[*a*]pyrene	2.5 µg/plate	TA98

3. Activation: The S9 was derived from male Sprague-Dawley rats (age, weight, and supplier were not reported)

[X]	Induced	[X]	Aroclor 1254	[X]	Rat	[X]	Liver
[ ]	Non-induced	[ ]	Phenobarbital	[ ]	Mouse	[ ]	Lung
[ ]		[ ]	β-naphthoflavone	[ ]	Hamster	[ ]	Other (*name*)
[ ]		[ ]	None	[ ]	Other (*name*)	[ ]	

The S9 fractions were purchased from MolTox Inc. (Boone, NC) and the average protein concentration was 38.2 mg/mL. It was not reported if the efficacy of the S9 fraction was checked before use. The 10% S9 mix contained: 1 mL S9 fraction, 0.2 mL of 0.25 M glucose-6-phosphate, 1 mL of 0.04 M NADP, 0.4 mL of 0.825 M KCl/0.2 M $MgCl_2$, 2.4 mL distilled water, and 5 mL of 0.2 M phosphate buffer at pH 7.4. The final S9 culture concentration was approximately 1.9%.

4. **Test organisms:** *S. typhimurium* and *E. coli* strains

[ ]	TA97	[X]	TA98	[X]	TA100	[ ]	TA102	[ ]	TA104
[X]	TA1535	[X]	TA1537	[ ]	TA1538	[X]	WP2*uvr*A	[ ]	WP2

Properly maintained?	[X]	Yes	[ ]	No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	[X]	Yes	[ ]	No

5. **Test compound concentrations used**

Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was not performed.

Mutagenicity assay: Tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A
Non-activated conditions: 0, 100, 333, 1000, 3333, or 5000 µg/plate

Activated conditions: 0, 100, 333, 1000, 3333, or 5000 µg/plate

In the mutagenicity assays, all concentrations of the test article, negative control, and positive controls were plated in triplicate, both in the presence and absence of S9-activation.

B. TEST PERFORMANCE

1. **Type of *Salmonella/E. coli* assay**

[X] Standard plate test
[ ] Pre-incubation __ minutes
[ ] "Prival" modification *(i.e. azo-reduction method)*
[ ] Spot test
[ ] Other

2. **Protocol:** *Salmonella* and *E. coli* tester strains were exposed to the test substance via the standard plate incorporation method. Bacteria (100 µL); test compound, solvent, or positive control (100 µL); and 0.5 mL of S9 mix (for tests requiring metabolic activation) or buffer were mixed with 2 mL of melted selective top agar and were poured into triplicate minimal glucose agar plates. After solidification of the top agar, the plates were inverted and incubated for 48 to 72 hours at 37±2EC. After incubation, the plates were scored for the number of revertant colonies either by an automated colony counter or manually. The plates were also checked for cytotoxicity (thinning of the background lawn) and test article precipitate.

3. **Statistical analysis:** Mean and standard deviation were calculated for each replicate.

4. **Evaluation criteria**

a. **Assay validity:** The assay was considered valid if the following criteria were met:
- All *S. typhimurium* tester strains exhibited sensitivity to crystal violet (*rfa* wall mutation),
- All tester strains exhibited sensitivity to ultraviolet light (*uvr*A or *uvr*B mutation),
- Strains TA98 and TA100 exhibited ampicillin resistance (pKM101 plasmid),

- The spontaneous reversion rates of all strains in the vehicle controls were within the historical control ranges,
- The positive controls induced at least a 3-fold increase in revertant colonies compared to the concurrent vehicle control, and
- A minimum of 3 non-toxic dose levels were available for evaluation.

b. Positive result: The test article was considered to be positive for mutagenicity if the following criteria were met:

- A dose-related, 2-fold or greater increase in the mean number of revertants/plate compared to solvent controls was observed in strains TA98, TA100, or WP2 *uvr*A
- A dose-related, 3-fold or greater increase in the mean number of revertants/plate compared to solvent controls was observed in strains TA1535 or TA1537

II. REPORTED RESULTS:

The test material formulations were not analyzed for actual concentrations.

A. PRELIMINARY CYTOTOXICITY ASSAY: A preliminary cytotoxicity assay was not performed.

B. MUTAGENICITY ASSAY: The results of the mutagenicity assay were presented in Tables 1-10 on pages 18-27 and were summarized in Tables 11 and 12 on page 28 of the study report. As the results of these assays were negative, Tables 11 and 12 are included as an Attachment to this DER. No precipitation of the test material was observed at any concentration. No evidence of treatment-related cytotoxicity (as indicated by thinning of background lawn or reduced number of revertants) was observed at any concentration in the presence or absence of S9-activation. There were no marked increases in the mean number of revertants/plate in any strain. The positive controls induced marked increases in revertant colonies compared to controls in all strains in the presence and absence of S9-activation.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS CONCLUSIONS: The investigators concluded that under the conditions of this study, IN-KJM44 did not induce mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, or *E. coli* strain WP2 *uvr*A when tested up to the limit dose (5000 µg/plate) in the presence or absence of S9-activation.

B. REVIEWER COMMENTS: No evidence of cytotoxicity (as indicated by thinning of background lawn or reduced number of revertants) was observed at any concentration in the presence or absence of S9-activation. There were no marked increases in the mean number of revertants/plate in any strain. The positive controls induced the appropriate response. **There was no evidence of induced mutant colonies over background.**

C. **STUDY DEFICIENCIES:** The following deficiencies were noted, but do not change the conclusions of this DER.

- A Quality Assurance statement was not provided.
- The test material formulations were not evaluated for actual concentrations

ATTACHMENT

The following attachment contains Tables 11 and 12 from page 28 of MRID 47560033.

Table 11: Summary of the mutagenicity test without rat liver S9

Dose	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
(µg/plate)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	12	10	100	19	9	4	3	1	32	5
positive control	43	4	998	77	571	41	687	18	999	45
100	5	3	101	14	6	3	1	2	37	5
333	6	3	99	12	4	3	4	1	33	5
1000	10	4	93	10	5	4	3	3	32	11
3333	8	3	100	14	2	2	2	2	26	7
5000	7	3	91	1	4	1	3	2	28	6

Experiment No: E1 & E-2
Plate Aliquot: 100 µL

Table 12: Summary of the mutagenicity test with rat liver S9

Dose	Number of Revertants Per Plate									
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
(µg/plate)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
vehicle	23	9	134	6	9	4	8	2	45	5
positive control	395	49	2204	148	154	31	121	13	410	41
100	32	4	113	3	9	2	4	2	47	13
333	26	6	111	5	9	1	10	3	41	1
1000	26	3	128	20	10	2	8	3	44	2
3333	26	5	104	5	8	5	13	5	51	7
5000	26	5	119	14	9	5	7	3	57	3

Experiment No: E-1
Plate Aliquot: 100 µL

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.5100 [§84-2]; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 5-1-209 J (MRID 47560019)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer: David A. McEwen, B.S.	Signature: David A. McEwen Date: 03/27/09
Secondary Reviewer: Ronnie J. Bever Jr., Ph.D.	Signature: Ronnie J. Bever Jr. Date: 03/27/09
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 03/27/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Steven Brecher Date: 03/27/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:** [signature]
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Reviewer: Marquea D. King, Ph.D. **Signature:** [signature]
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** [signature]
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Bacterial Gene Mutation *(Salmonella typhimurium)*/ mammalian activation gene mutation assay; OPPTS 870.5100 [§ 84-2]; OECD 471.

PC CODES: 288008 **DP BARCODE:** D361080
TXR #: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Wagner III, V.O. and M.R. VanDyke. (2007) DPX-MAT28 technical: Bacterial reverse mutation assay. BioReliance, Rockville, MD. Laboratory Study No.: AC02HJ.503.BTL, October 5, 2007. MRID 47560019. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Newark, DE

EXECUTIVE SUMMARY: In two independent trials of a reverse gene mutation assay in bacteria (MRID 47560019), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvr*A were exposed to DPX-MAT28 (Amino-cyclopyrachlor, 92.2% a.i., Batch No.: DPX-MAT28-009) in DMSO at concentrations of 0, 1.5, 5.0, 15, 50, 150, 500, 1500, or 5000 µg/plate (+/-S9, Trial 1) and 0, 50, 150, 500, 1500, or 5000 µg/plate (+/-S9, Trial 2) using the standard plate incorporation method. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor® 1254. Standard strain-specific mutagens served as positive controls.

DPX-MAT28 was tested up to the limit dose (5000 µg/plate). No evidence of cytotoxicity was observed at any concentration in any strain in either trial in the presence or absence of S9-activation. There were no marked increases in the mean number of revertants/plate in any strain. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. **There was no evidence of induced mutant colonies over background.**

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

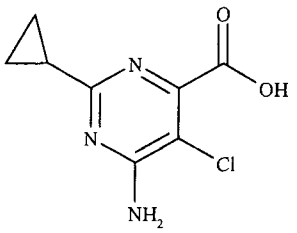
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28

Description: Solid
Batch #: DPX-MAT28-009
Purity: 92.2% a.i. (doses adjusted for purity)
Stability: Not reported
CAS # of TGAI: 858956-08-8
Structure:



2. Control materials

Negative: The solvent alone served as the negative control.
Solvent: Dimethylsulfoxide (DMSO; 50 µL/plate)
Positive:

Non-activation	Concentration	Strain
Sodium azide	1.0 µg/plate	TA100, TA1535
2-Nitrofluorene	1.0 µg/plate	TA98
9-Aminoacridine	75 µg/plate	TA1537
Methyl methanesulfonate	1000 µg/plate	WP2 *uvr*A
Activation		
2-Aminoanthracene	1.0 µg/plate	TA98, TA100, TA1535, and TA1537
	10 µg/plate	WP2 *uvr*A

3. Activation: The S9 was derived from male Sprague-Dawley rats (age, weight, and supplier not reported).

[X]	Induced	[X]	Aroclor 1254	[X]	Rat	[X]	Liver
[ ]	Non-induced	[ ]	Phenobarbital	[ ]	Mouse	[ ]	Lung
[ ]		[ ]	β-naphthoflavone	[ ]	Hamster	[ ]	Other (*name*)
[ ]		[ ]	None	[ ]	Other (*name*)	[ ]	

The S9 fractions were purchased from MolTox Inc. (Boone, NC). It was reported that the efficacy of the S9 fraction was checked before use. The S9 mix contained: 10% S9 fraction, 5 mM glucose-6-phosphate, 4 mM NADP, 8 mM $MgCl_2$, and 33 mM KCl in a 100 mM phosphate buffer at pH 7.4. The final S9 culture concentration was approximately 2%.

4. Test organisms: *S. typhimurium* and *E. coli* strains

[ ]	TA97	[X]	TA98	[X]	TA100	[ ]	TA102	[ ]	TA104
[X]	TA1535	[X]	TA1537	[ ]	TA1538	[X]	WP2*uvr*A	[ ]	WP2

Properly maintained?	[X]	Yes	[ ]	No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	[X]	Yes	[ ]	No

5. Test compound concentrations used

Preliminary cytotoxicity assay: The preliminary cytotoxicity assay served as Trial 1 of the mutagenicity assay (concentrations presented below).

Mutagenicity assay: Tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A
Non-activated conditions: 0, 1.5, 5.0, 15, 50, 150, 500, 1500, or 5000 µg/plate (Trial 1)
0, 50, 150, 500, 1500, or 5000 µg/plate (Trial 2)

Activated conditions: 0, 1.5, 5.0, 15, 50, 150, 500, 1500, or 5000 µg/plate (Trial 1)
0, 50, 150, 500, 1500, or 5000 µg/plate (Trial 2)

In the mutagenicity assays, all concentrations of the test article, negative control, and positive controls were plated in duplicate (Trial 1) or triplicate (Trial 2), both in the presence and absence of S9-activation.

B. TEST PERFORMANCE

1. Type of *Salmonella/E. coli* assay

- [X] Standard plate test
- [ ] Pre-incubation __ minutes
- [ ] "Prival" modification *(i.e. azo-reduction method)*
- [ ] Spot test
- [ ] Other

2. **Protocol:** *Salmonella* and *E. coli* tester strains were exposed to the test substance via the standard plate incorporation method. Bacteria (100 µL); test compound, solvent, or positive control (50 µL); and 0.5 mL of S9 mix (for tests requiring metabolic activation) or buffer were mixed with 2 mL of melted selective top agar and were poured into duplicate (Trial 1) or triplicate (Trial 2) plates containing 25 mL Vogel Bonner minimal medium E. After solidification of the top agar, the plates were inverted and incubated for 48 to 72 hours at 37±2EC. After incubation, the plates were scored for the number of revertant colonies either by an automated colony counter or manually. The plates were also checked for cytotoxicity (thinning of the background lawn) and test article precipitate.

3. **Statistical analysis:** Mean and standard deviation were calculated for each replicate.

4. **Evaluation criteria**

a. **Assay validity:** The assay was considered valid if the following criteria were met:
- All *S. typhimurium* tester strains exhibited sensitivity to crystal violet (*rfa* wall mutation),
- All tester strains exhibited sensitivity to ultraviolet light (*S. typhimurium* strains, *uvr*B mutation and *E. coli* strain, *uvr*A mutation),
- Strains TA98 and TA100 exhibited ampicillin resistance (pKM101 plasmid),
- The spontaneous reversion rates of all strains in the vehicle controls were within the historical control ranges,

- The positive controls induced at least a 3-fold increase in revertant colonies compared to the concurrent vehicle control, and
- A minimum of 3 non-toxic dose levels were available for evaluation.

b. **Positive result:** The test article was considered to be positive for mutagenicity if the following criteria were met:
- A dose-related, 2-fold or greater increase in the mean number of revertants/plate compared to solvent controls was observed in strains TA98, TA100, or WP2 *uvr*A
- A dose-related, 3-fold or greater increase in the mean number of revertants/plate compared to solvent controls was observed in strains TA1535 or TA1537

II. REPORTED RESULTS: The test material formulations were analyzed for actual concentrations using HPLC and were 89.6-100.8% of nominal.

A. **PRELIMINARY CYTOTOXICITY ASSAY:** The preliminary cytotoxicity assay served as Trial 1 of the mutagenicity assay (data presented below).

B. **MUTAGENICITY ASSAY:** The results of the mutagenicity assay were presented in Tables 1-20 on pages 22-41 and were summarized in Tables 21 and 22 on pages 42 and 43 of the study report. As the results of these assays were negative, Tables 21 and 22 are included as an Attachment to this DER. No precipitation of the test material was observed at any concentration in either trial. No evidence of treatment-related cytotoxicity (as indicated by thinning of background lawn or reduced number of revertants) was observed at any concentration in either trial in the presence or absence of S9-activation. There were no marked increases in the mean number of revertants/plate in any strain in either trial. The positive controls induced marked increases in revertant colonies compared to controls in all strains in the presence and absence of S9-activation in both trials.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** The investigators concluded that under the conditions of this study, DPX-MAT28 did not induce mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, or *E. coli* strain WP2*uvr*A when tested up to the limit dose (5000 µg/plate) in the presence or absence of S9-activation.

B. **REVIEWER COMMENTS:** No evidence of cytotoxicity (as indicated by thinning of background lawn or reduced number of revertants) was observed at any concentration in either trial in the presence or absence of S9-activation. There were no marked increases in the mean number of revertants/plate in any strain in either trail. The positive controls induced the appropriate response in both trials. **There was no evidence of induced mutant colonies over background.**

C. **STUDY DEFICIENCIES:** None

ATTACHMENT

The following attachment contains Tables 21 and 22 from pages 42 and 43 of MRID 47560019.

Table 21

Test Article Id : DPX-MAT28
Study Number : AC02HJ.503.BTL Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Vehicle	28 ± 4	179 ± 25	19 ± 6	8 ± 1	34 ± 7
1.5	25 ± 11	195 ± 6	22 ± 1	9 ± 1	30 ± 6
5.0	22 ± 0	181 ± 27	21 ± 6	8 ± 1	24 ± 4
15	24 ± 1	210 ± 13	20 ± 3	11 ± 1	27 ± 6
50	19 ± 2	154 ± 25	22 ± 1	5 ± 1	32 ± 5
150	11 ± 1	184 ± 8	21 ± 4	6 ± 1	31 ± 7
500	23 ± 7	228 ± 23	20 ± 3	14 ± 1	34 ± 2
1500	24 ± 4	204 ± 16	18 ± 2	9 ± 4	35 ± 4
5000	23 ± 2	186 ± 9	19 ± 2	7 ± 3	32 ± 0
Positive	283 ± 42	717 ± 129	729 ± 148	794 ± 69	439 ± 83

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Vehicle	26 ± 3	201 ± 47	16 ± 3	10 ± 2	36 ± 8
1.5	19 ± 4	203 ± 13	17 ± 2	11 ± 1	39 ± 4
5.0	24 ± 6	226 ± 9	19 ± 2	11 ± 3	36 ± 3
15	25 ± 6	194 ± 15	18 ± 1	10 ± 4	44 ± 1
50	20 ± 0	217 ± 24	18 ± 4	12 ± 4	44 ± 7
150	19 ± 4	211 ± 33	25 ± 2	8 ± 6	34 ± 3
500	26 ± 11	199 ± 30	21 ± 1	8 ± 2	34 ± 1
1500	25 ± 4	187 ± 1	15 ± 3	9 ± 4	41 ± 6
5000	35 ± 5	201 ± 1	19 ± 3	9 ± 2	41 ± 4
Positive	666 ± 134	1190 ± 329	154 ± 13	150 ± 14	353 ± 97

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 50 µL

Table 22

Test Article Id : DPX-MAT28
Study Number : AC02HJ.503.BTL Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Vehicle	14 ± 4	124 ± 11	10 ± 5	8 ± 4	26 ± 3
50	16 ± 7	143 ± 5	13 ± 5	5 ± 3	25 ± 8
150	17 ± 2	194 ± 28	13 ± 3	5 ± 1	16 ± 4
500	12 ± 2	136 ± 27	12 ± 5	6 ± 2	26 ± 2
1500	15 ± 3	153 ± 2	10 ± 3	6 ± 2	17 ± 2
5000	17 ± 3	130 ± 8	13 ± 1	7 ± 1	28 ± 9
Positive	239 ± 44	573 ± 21	512 ± 35	800 ± 97	346 ± 7

Liver Microsomes: Rat liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvrA*
Vehicle	25 ± 4	179 ± 12	13 ± 6	9 ± 2	24 ± 3
50	21 ± 8	167 ± 12	8 ± 2	9 ± 3	29 ± 6
150	24 ± 3	162 ± 15	10 ± 4	6 ± 2	35 ± 4
500	22 ± 5	148 ± 23	12 ± 4	7 ± 2	36 ± 7
1500	21 ± 2	152 ± 13	11 ± 3	9 ± 1	34 ± 2
5000	21 ± 3	172 ± 10	12 ± 5	10 ± 3	31 ± 4
Positive	307 ± 53	725 ± 238	114 ± 16	64 ± 5	247 ± 10

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot: 50 µL

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.5300 [§84-2]; CHO Cells/Mammalian Activation Gene Forward Mutation Assay at the HGPRT Locus

Work Assignment No. 5-1-209 K (MRID 47560020)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
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Prepared by

Pesticides Health Effects Group
Sciences Division
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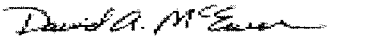
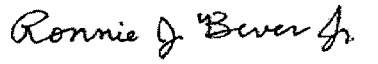
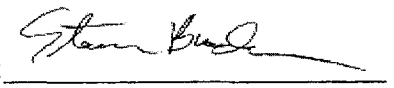
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer: David A. McEwen, B.S.	Signature:  Date: 03/27/09
Secondary Reviewer: Ronnie J. Bever Jr., Ph.D.	Signature:  Date: 03/27/09
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: 03/27/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature:  Date: 03/27/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman **Signature:**
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Reviewer: Marquea D. King, Ph.D. **Signature:**
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cell Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODES: 288008 **DP BARCODE:** D361080
TXR #: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Clarke, J.J. (2007) DPX-MAT28 technical: In vitro mammalian cell gene mutation test (CHO/HGPRT assay). BioReliance, Rockville, MD. Laboratory Study No.: AC02HJ.782.BTL, October 9, 2007. MRID 47560020. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Newark, DE

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the HGPRT locus (MRID 47560020), duplicate cultures of Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to DPX-MAT28 (Aminocyclopyrachlor, 92.2% a.i., doses adjusted for purity, Batch No: DPX-MAT28-009) in DMSO at concentrations of 0, 750, 1000, 1500, 1750, or 2150 µg/mL in the presence and absence of S9-activation for 5 hours.

DPX-MAT28 was tested up to 2150 µg/mL, which was the solubility limit (4000 µg/mL +/-S9 was precipitating). No precipitation of the test material or evidence of cytotoxicity were observed at any concentration in the presence or absence of S9. No marked increase in mutant frequency was observed at any concentration in the presence or absence of S9-activation. The positive controls induced the appropriate response in the presence and absence of S9. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

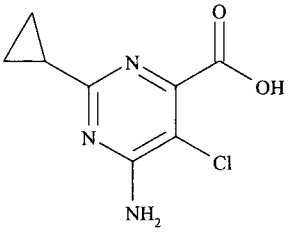
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28

Description: White powder
Batch #: DPX-MAT28-009
Purity: 92.2% a.i. (doses adjusted for purity)
CAS # of TGAI: 858956-08-8
Structure:



2. Control materials

Negative control: The solvent control alone served as the negative control.
Solvent control: DMSO (1% v/v)
Positive control: Non-activation: Ethyl methanesulfonate (EMS, 0.2 µL/mL)
Activation: Benzo(a)pyrene (BaP, 4 µg/mL)

3. Activation: The S9 was derived from male rats (strain, age, weight, and supplier were not reported).

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			None		Hamster		Other
			Other		Other		

The S9 fraction was purchased from MolTox, Inc. (Boone, NC) and was stored at ≤ -60 EC until used. The protein content was not reported; however, the efficacy of the batch was checked prior to use. The S9 mix consisted of 0.1 mL S9 fraction/mL cofactor pool (4 mM NADP, 10 mM $MgCl_2$, 30 mM KCl, 5 mM glucose-6-phosphate, and 50 mM sodium phosphate buffer, pH 8.0). The final S9 culture concentration was approximately 2%.

4. Test cells: Mammalian cells in culture

	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
X	Chinese hamster ovary (CHO) cells		list any others

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?		Yes	X	No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

Media: The culture medium contained hypoxanthine-free Ham's F12 medium supplemented with 5% dialyzed fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 µg/mL), and 2 mM L-glutamine/mL. The treatment medium was the same as the culture medium. The selection medium was culture medium containing 6-thioguanine (10 µM).

5. Locus examined

	☐ **Thymidine kinase (TK)**	☒	**Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)**	☐	**Na^+/K^+ ATPase**
Selection agent:	☐ Bromodeoxyuridine (BrdU)	☐	8-azaguanine (8-AG)	☐	Ouabain
	☐ Fluorodeoxyuridine (FdU)	☒	6-thioguanine (6-TG; 10 µM)		
	☐ Trifluorothymidine (TFT)				

6. Test compound concentrations used

a. Preliminary cytotoxicity (triplicate plates)
Non-activated conditions: 0, 0.5, 1.5, 5, 15, 50, 140, 460, 1370, and 4000 µg/mL
Activated conditions: 0, 0.5, 1.5, 5, 15, 50, 140, 460, 1370, and 4000 µg/mL
The concentration of 4000 µg/mL was a precipitating concentration.

b. Mutagenicity assay (duplicate plates)
Non-activated conditions: 0, 750, 1000, 1500, 1750, and 2150 µg/mL
Activated conditions: 0, 750, 1000, 1500, 1750, and 2150 µg/mL
The concentration of 2150 µg/mL was 10 mM (limit dose for solubility).

B. TEST PERFORMANCE

1. Cell treatment

a. Cells were exposed to test compound, solvent or positive controls for 5 hours (non-activated) and 5 hours (activated).

b. After washing, cells were cultured for 7-9 days (with subculturing at 2-4 days; expression period) before cell selection.

c. After expression, $2x10^5$ cells/dish (5 dishes/group) were cultured for 7-10 days in selection medium to determine numbers of mutants and 100 cells/dish (3 dishes/group) were cultured for 7-10 days without selective agent to determine cloning efficiency.

2. Statistical methods: Statistical analysis of the data was not performed, nor required.

3. Evaluation criteria

a. Assay validity: The assay was considered valid if the following criteria were met:
- The cloning efficiency of the vehicle control was >50% and the spontaneous mutant frequency was within 0-25 mutants/10^6 clonable cells,
- The positive control must induce an average mutant frequency of at least 3x the vehicle control and must exceed 40 mutants/10^6 clonable cells,
- At least 4 analyzable test concentrations showing mutants were available.

b. Positive result: The test article was considered mutagenic if:
- A mutant frequency of >40 mutants/10^6 clonable cells was observed at two or more consecutive concentrations, and

The observed increased mutant frequencies were concentration-dependent.

II. REPORTED RESULTS: The dose formulations were analyzed for homogeneity and actual concentrations using HPLC. The 100 and 215 mg/mL dose formulations were homogeneous (2.42-6.43% RSD) and the actual concentrations of the 50, 100, and 215 mg/mL dose formulations were within 99.1-115.3% of nominal. Stability of the 0.03 and 215 mg/mL dose formulations, held at room temperature for 14 hours, was verified (100.5% nominal).

A. **PRELIMINARY CYTOTOXICITY ASSAY:** Precipitation of the test material was observed at 4000 µg/mL. No relevant cytotoxicity was observed at any concentration after 5 hours of treatment in the presence or absence of S9-activation. The 10 mM limit dose was 2150 µg/mL. Based on these results, concentrations of 0, 1000, 1500, 1750, and 2150 µg/mL (±S9) were selected for the mutagenicity assay.

B. **MUTAGENICITY ASSAY:** The results of the mutagenicity assays were presented in Study Report Tables 3 and 4 on pages 19-20 and are included as an Attachment to this DER.

DPX-MAT28 was tested up to 2150 µg/mL (limit dose, 10 mM, +/-S9). No precipitation of the test material or evidence of cytotoxicity were observed at any concentration in the presence or absence of S9. No marked increase in mutant frequency was observed at any concentration in the presence or absence of S9-activation. The positive controls (EMS, -S9 and BaP, +S9) induced substantial increases in mutation frequency.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** The investigators concluded that DPX-MAT28 did not induce mutation at the HGPRT locus of CHO cells at up to 2150 µg/mL (limit dose of solubility, +/-S9).

B. **REVIEWER COMMENTS:** DPX-MAT28 was tested up to 2150 µg/mL (limit dose of solubility, 10 mM, +/-S9). No precipitation of the test material or evidence of cytotoxicity were observed at any concentration in the presence or absence of S9. No marked increase in mutant frequency was observed at any concentration in the presence or absence of S9-activation. The positive controls induced the appropriate response in the presence and absence of S9. **There was no evidence of induced mutant colonies over background in the presence or absence of S9-activation.**

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

C. **STUDY DEFICIENCIES:** None

ATTACHMENT

The following attachment contains Tables 3 and 4 from pages 19 and 20 of MRID 47560020.

TABLE 3

Non-activated (-S9) Study using DPX-MAT28

Cloning Efficiency Plates						Cloning Efficiency	Selection (Mutation) Plates						Mutants/10^6 Clonable Cells
Treatment (µg/mL)	Subset	Plate Counts			Average Colonies		Plate Counts					Average Colonies	
		1	2	3			1	2	3	4	5		
Solvent	A	115	97	120	85.8	0.86	0	0	0	0	0	0	**0**
	B	70	62	51			0	0	0	0	0		
EMS (0.2µL/mL)	A	66	60	86	72.5	0.73	47	43	37	40	42	38.6	**266.2**
	B	60	76	87			38	36	35	34	34		
750	A	96	97	72	76.0	0.76	0	0	0	2	1	0.3	**2.0**
	B	65	58	68			0	0	0	0	0		
1000	A	98	86	88	90.3	0.90	8	5	5	3	4	3.2	**17.7**
	B	78	83	109			0	2	0	4	1		
1500	A	94	77	106	78.5	0.79	0	0	0	0	0	0	**0**
	B	79	49	66			0	0	0	0	0		
1750	A	65	67	63	80.7	0.81	0	0	0	0	0	0.9	**5.6**
	B	91	102	96			4	0	1	2	2		
2150	A	90	60	92	72.7	0.73	0	0	0	0	0	0.7	**4.8**
	B	73	64	57			1	1	1	1	3		

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{100 cells/dish}}$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x } 10^5\text{ cells}} \times 10^6$$

A and B are duplicate cultures

TABLE 4

Activated (+S9) Study using DPX-MAT28

Cloning Efficiency Plates						Cloning Efficiency	Selection (Mutation) Plates						Mutants/10^6 Clonable Cells
Treatment (µg/mL)	Subset	Plate Counts			Average Colonies		Plate Counts					Average Colonies	
		1	2	3			1	2	3	4	5		
Solvent	A	97	96	73	79.2	0.79	0	0	0	0	0	0	**0**
	B	55	69	85			0	0	0	0	0		
B(a)P (4µg/mL)	A	80	63	72	71.0	0.71	21	15	16	14	22	24.3	**171.1**
	B	57	60	94			22	42	19	26	46		
750	A	110	106	98	97.5	0.98	0	0	1	4	0	0.5	**2.6**
	B	92	94	85			0	0	0	0	0		
1000	A	62	59	73	65.8	0.66	0	0	0	0	0	0.3	**2.3**
	B	57	46	98			2	0	1	0	0		
1500	A	76	64	95	69.2	0.69	0	0	0	0	1	0.2	**1.4**
	B	55	66	59			0	0	1	0	0		
1750	A	62	94	92	84.0	0.84	3	2	4	4	2	2.1	**12.5**
	B	74	86	96			0	2	2	1	1		
2150	A	84	111	86	80.2	0.80	0	1	1	2	2	1.0	**6.2**
	B	45	69	86			0	0	1	1	2		

$$\text{Cloning efficiency} = \frac{\text{average colonies}}{\text{100 cells/dish}}$$

$$\text{Mutants/}10^6\text{ clonable cells} = \frac{\text{average mutant colonies}}{\text{cloning efficiency X 2 x } 10^5\text{ cells}} \times 10^6$$

A and B are duplicate cultures

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.5375 [§84-2]; *In Vitro* Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes

Work Assignment No. 5-1-209 L (MRID 47560021)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
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Prepared by

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Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.
Signature: Steven Brecher
Date: 3/27/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Reviewer: Marquea D. King, Ph.D. **Signature:**
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cytogenetics (Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes) OPPTS 870.5375 [§84-2]; OECD 473.

PC CODES: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Gudi, R. and M. Rao. (2007) DPX-MAT28 technical: *In vitro* mammalian chromosome aberration test in human peripheral blood lymphocytes. BioReliance, Rockville, MD. Laboratory Study No.: AC02HJ.341.BTL, October 5, 2007. MRID 47560021. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Newark, DE

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay (chromosome aberration; MRID 47560021), lymphocyte cultures were prepared from human peripheral blood and exposed to DPX-MAT28 (92.2% a.i., doses adjusted for purity, Batch # DPX-MAT28-009) in DMSO at concentrations of 0, 267, 534, 1068, or 2136 µg/mL for either 4 hours followed by a 16 hour recovery period (+/-S9) or for 20 hours with no recovery period (-S9). The S9 fraction was derived from the livers of male rats induced with Aroclor 1254. Cyclophosphamide and mitomycin C served as the positive controls in the presence and absence of S9, respectively.

DPX-MAT28 was tested up to the limit dose (2136 µg/mL, 10mM). No precipitation of the test material was observed at any concentration (+/-S9). No substantial decreases in mitotic index were observed at any concentration after 4 (+/-S9) or 20 hrs (-S9) of treatment; therefore, concentrations of 0, 534, 1068, and 2136 µg/mL were selected for evaluation of chromosomal aberrations in each assay. No significant increases in the mean percentage of cells with structural or numerical abortions or in aberrations per cell were observed after treatment for 4 hrs (+/-S9) or 20 hrs (-S9). The positive controls induced the appropriate response in the presence and absence of S9. **There was no evidence of chromosome aberrations induced over background in the presence or absence of S9-activation.**

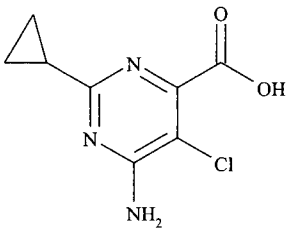
This study is classified as **Acceptable/Guideline** and satisfies the Guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* mutagenicity (chromosome aberration) data. **COMPLIANCE:** Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28

Description: White powder
Batch #: DPX-MAT28-009
Purity: 92.2% a.i. (doses adjusted for purity)
CAS # of TGAI: 858956-08-8
Structure:



2. Control materials

Negative control: The solvent alone served as the negative control.
Solvent control: DMSO (1% v/v)
Positive control: Non-activated: Mitomycin C (0.6 and 0.3 µg/mL, 4 and 20 hr exposures, respectively)
Activated: Cyclophosphamide (20 µg/mL)

3. Activation: The S9 fraction was derived from male rats (strain, age, weight, and supplier not reported).

X	Induced	X	Aroclor™ 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			β-naphthoflavone		Hamster		Other
			Other		Other		

The S9 fraction was purchased from MolTox, Inc. (Boone, NC). The storage conditions and protein content were not reported; however, it was stated that the efficacy of the S9 fraction was checked before use. The S9 fraction was thawed prior to use and was mixed with a cofactor pool. The S9 mix contained the following per mL of medium (RPMI serum-free medium supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine): 1 mM glucose-6-phosphate, 1 mM NADP, 2 mM $MgCl_2$, 6 mM KCl , and 20 µL S9 fraction. The final S9 culture concentration was approximately 1.6%.

4. Test cells: Human blood samples were obtained from healthy, non-smoking female donors (one each day) on the days of culture initiation. Each donor had no recent history of radiotherapy, viral infection, or the administration of drugs.

	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells	X	Human lymphocytes

Media: RPMI 1640 complete medium (% serum not reported) supplemented with 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin.

Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination? **Not applicable**		Yes		No

Periodically checked for karyotype stability? **Not applicable** ☐ Yes ☐ No

Whole blood cultures were established by placing 0.6 mL blood in 9.4 mL RPMI complete medium. Phytohemagglutinin (1% v/v) was added to stimulate the lymphocytes to divide, and the cultures were incubated at 37±1°C in a humidified 5±1% CO_2 atmosphere for 44-48 hours.

5. Test compound concentrations used:

Non-activated conditions:	Preliminary toxicity: 0, 0.21, 0.64, 2.14, 6.41, 21.36, 64.08, 213.6, 640.8, and 2136 µg/mL (4 hr and 20 hr exposures)
	Mutagenicity Assay: 0, 267, 534, 1068, and 2136 µg/mL (4 hr and 20 hr exposures; Duplicate cultures)
Activated conditions:	Preliminary toxicity: 0, 0.21, 0.64, 2.14, 6.41, 21.36, 64.08, 213.6, 640.8, and 2136 µg/mL (4 hr exposure)
	Mutagenicity Assay: 0, 267, 534, 1068, and 2136 µg/mL (4 hr exposure; Duplicate cultures)

B. TEST PERFORMANCE

1. **Preliminary cytotoxicity assay:** The preliminary cytotoxicity assay was performed using the same methods reported below.

2. **Cytogenetic assay:** Approximately 44-48 hours after culture establishment, 0.1 mL of the test material, solvent control, or positive control were added to duplicate blood cultures. Treatment was carried out in 10 mL of S9 reaction mixture (8 mL serum-free medium and 2 mL S9 mix) for the activated cultures or 10 mL of fresh complete medium in the non-activated cultures. The cultures were incubated at 37±1°C in a humidified 5±1% CO_2 atmosphere for either 4 hrs (+/-S9) or 20 hrs (-S9). After the 4 hr exposure period, the treatment medium was removed. The cells were then washed with Ca and Mg-free phosphate buffered saline (PBS), fed complete medium, and returned to the incubator for an additional 16 hrs. In the 20 hr exposure group, treatment was continuous until the time of harvest. Approximately 2 hrs prior to collection, the cultures were treated with Colcemid® (0.1 mL).

a. Cell exposure time	Test material	Solvent control	Positive control
Non-activated:	4 hrs 20 hrs	4 hrs 20 hrs	4 hrs 20 hrs
Activated:	4 hrs	4 hrs	4 hrs

b. Spindle inhibition	
Inhibition used/concentration:	Colcemid® (0.1 µg/mL)
Administration time:	2 hours (before cell harvest)

c. Cell harvest time after initiation of treatment	Test material	Solvent control	Positive control
Non-activated:	20 hrs 20 hrs	20 hrs 20 hrs	20 hrs 20 hrs
Activated:	20 hrs	20 hrs	20 hrs

d. Details of slide preparation: After cell division was arrested, the cultures were centrifuged, the supernatant was removed, and the cells were resuspended in 5 mL of 0.075 M KCl and incubated at 37±1°C for 20 minutes. The cultures were gently mixed and 0.5 mL of fixative (methanol/glacial acetic acid, 3:1 v/v) was added. The cultures were centrifuged, the supernatant was removed, the cells were fixed in two washes of methanol/glacial acetic acid (3:1 v/v), and stored in fixative at least overnight at approximately 2-8°C. The cultures were centrifuged, most of the supernatant was removed, and one or two drops of the lymphocyte suspension were dropped onto clean microscope slides. The slides were air-dried, stained with 5% Giemsa, air-dried, and permanently mounted with coverslips.

e. Metaphase analysis

No. of cells examined per dose: 200 cells (100/replicate) in the treatment and solvent control groups were scored for structural and numerical aberrations. In the positive control groups, 200 cells (100/replicate) were scored for numerical aberrations and 100 cells (50/replicate) were scored for structural aberrations. The mitotic index was recorded as the % of cells in mitosis per 500 cells counted.

Scored for structural?	[X]	Yes	[ ]	No
Scored for numerical?	[X]	Yes (polyploidy and endoreduplication)	[ ]	No
Coded prior to analysis?	[X]	Yes	[ ]	No

f. Evaluation criteria

Assay validity: The assay was considered valid if the following criteria were met:

- The frequency of cells with structural chromosome aberrations in the solvent control was within the historical control range (for this study the historical ranges were 0 to 0.5%, +S9 and 0 to 1.0%, -S9), and
- The frequency of cells with structural chromosome aberrations in the positive controls must be significantly ($p \leq 0.05$) increased relative to the solvent control.

Positive result: The test article was considered to be mutagenic if the following criteria were met:

- A statistically significant increase in the number of cells with structural aberrations was observed at one or more concentrations in a dose-related manner, and
- The increase in aberrations was substantially higher than the historical negative control range.

g. Statistical analysis: The frequencies of aberrant cells were analyzed using Fisher's Exact test ($p \leq 0.05$). In the event of a positive result, the Cochran-Armitage test was used to measure dose-responsiveness.

II. REPORTED RESULTS: The dose formulations were analyzed for actual concentrations using HPLC, and were within 98.5-114% of nominal. Addition of the test material had no significant effect on the osmolality or pH of the culture media. No precipitation of the test material was observed at any concentration. The test material was tested at up to the 10 mM limit dose (2136 µg/mL).

A. **PRELIMINARY CYTOTOXICITY ASSAY:** In the preliminary cytotoxicity assay, substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) was not observed at any concentration in any treatment group. Therefore, concentrations of 0, 267, 534, 1068, and 2136 µg/mL (±S9) were selected for the cytogenetic assays.

B. **CYTOGENETIC ASSAY:** The results of the cytogenetic assays were presented in the Tables 4-6 on pages 24-26 and were summarized in Table 7 on page 27, which is included as an Attachment to this DER.

No substantial decreases in mitotic index were observed at any concentration after 4 (+/-S9) or 20 hrs (-S9); therefore, concentrations of 0, 534, 1068, and 2136 µg/mL were selected for evaluation of chromosomal aberrations in each assay. No significant increases in the mean percentage of cells with structural or numerical aberrations or in aberrations per cell were observed after treatment for 4 hrs (+/-S9) or 20 hrs (-S9). The positive controls induced increases ($p \leq 0.01$) in the mean percent aberrant cells in all groups.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** The investigators concluded that DPX-MAT28 did not induce chromosome aberrations in cultured human peripheral blood lymphocytes at up to 2136 µg/mL (10 mM, limit dose) in the presence or absence of S9-activation.

B. **REVIEWER COMMENTS:** No significant increases in the mean percentage of aberrant cells were observed in the presence or absence of S9. The positive controls induced increases ($p \leq 0.01$) in the number of aberrant cells in the presence and absence of S9 in all groups. **There was no evidence of chromosome aberrations induced over background in the presence or absence of S9-activation.**

C. **STUDY DEFICIENCIES:** The following minor deficiency was noted, but does not change the conclusions of this DER: The % serum in the complete culture medium was not reported.

ATTACHMENT

The following attachment contains Table 7 from page 27 of MRID 47560021.

TABLE 7
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	10.3	200	200	0.000	±0.000	0.0	0.0
DPX-MAT28									
534	-S9	4	8.3	200	200	0.000	±0.000	0.0	0.0
1068	-S9	4	7.8	200	200	0.000	±0.000	0.0	0.0
2136	-S9	4	9.5	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	5.7	200	100	0.240	±0.534	0.0	19.0**
DMSO	+S9	4	9.5	200	200	0.000	±0.000	0.0	0.0
DPX-MAT28									
534	+S9	4	9.4	200	200	0.005	±0.071	0.0	0.5
1068	+S9	4	10.0	200	200	0.000	±0.000	0.0	0.0
2136	+S9	4	10.3	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	5.5	200	100	0.230	±0.446	0.0	22.0**
DMSO	-S9	20	10.3	200	200	0.005	±0.071	0.0	0.5
DPX-MAT28									
534	-S9	20	8.2	200	200	0.000	±0.000	0.0	0.0
1068	-S9	20	8.6	200	200	0.000	±0.000	0.0	0.0
2136	-S9	20	4.7	200	200	0.005	±0.071	0.0	0.5
MMC, 0.3	-S9	20	5.9	200	100	0.210	±0.409	0.0	21.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's Exact test.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.5395 [§84-2]; Micronucleus Assay in Mice

Work Assignment No. 5-1-209 M (MRID 47560022)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
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Durham, NC 27713

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Secondary Reviewer: Ronnie J. Bever Jr., Ph.D.	Signature: Ronnie J Bever Jr. Date: 03/27/09
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 03/27/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Steven Brecher Date: 03/27/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Reviewer: Marquea D. King, Ph.D. **Signature:**
Re-registration Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

PC CODES: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Krsmanovic, L. and T. Huston. (2007) DPX-MAT28 technical: Mouse bone marrow erythrocyte micronucleus test. BioReliance, Rockville, MD. Laboratory Study No.: AC02HJ.123.BTL, October 5, 2007. MRID 47560022. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Newark, DE

EXECUTIVE SUMMARY: In a bone marrow micronucleus assay (MRID 47560022), 5 young adult Crl:CD1(ICR) mice/sex/dose/sampling time were treated once via gavage (10 mL/kg) with DPX-MAT28 (Aminocyclopyrachlor; 92.2% a.i., doses adjusted for purity; Batch No. DPX-MAT28-009) in sterile water at doses of 0, 500, 1000, or 2000 mg/kg (limit dose). Bone marrow cells were harvested at 24 and 48 hours post-dosing. Additionally, 5 mice/sex were treated with cyclophosphamide (50 mg/kg) and marrow cells were harvested at 24 hours post-dosing.

DPX-MAT28 was tested at up to the limit dose (2000 mg/kg). No mortality or clinical signs of toxicity were observed at any dose at either time point. No significant decrease in polychromatic erythrocyte to normochromatic erythrocyte ratios (PCE:NCE) was observed, indicating that the test material was not toxic to the bone marrow. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

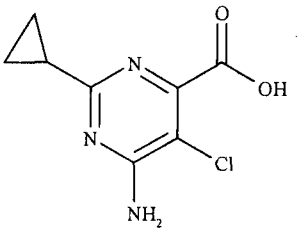
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28

Description: White powder
Batch #: DPX-MAT28-009
Purity: 92.2% a.i. (doses adjusted for purity)
CAS # of TGAI: 858956-08-8
Structure:



2. Control materials

Negative: The vehicle alone served as the negative control
Vehicle: Sterile water (10 mL/kg)
Positive: Cyclophosphamide (CP, 50 mg/kg at a volume of 10 mL/kg)

3. Test animals

Species:	Mouse			
Strain:	Crl:CD1(ICR)			
Age/weight at study initiation:	Approximately 6-8 weeks; 25.8-29.7 g males and 20.8-25.7 g females			
Source:	Charles River Laboratories, Inc. (Raleigh, NC)			
No. of animals used per dose/time point:	[5] Males		[5] Females	
Properly maintained?	[X] Yes		[ ] No	

4. Test compound administration

	Dose levels	Final volume	Route
Range-finding study	1, 10, 100, 1000, and 2000 mg/kg	10 mL/kg	Gavage
Main study	0, 500, 1000, and 2000 mg/kg	10 mL/kg	Gavage

B. TEST PERFORMANCE

1. Treatment and sampling times

a. Test compound and vehicle control

Dosing:	[X] once		[ ] twice (24 hrs apart)			[ ] Other			
Sampling (after last dose):	[ ] 6 hr		[ ] 12 hr	[X] 24 hr		[X] 48 hr		[ ] 72 hr	

b. Positive control

Dosing:	[X] once		[ ] twice (24 hrs apart)			[ ] Other			
Sampling (after last dose):	[ ] 6 hr		[ ] 12 hr	[X] 24 hr		[ ] 48 hr		[ ] 72 hr	

2. Tissues and cells examined

Bone marrow	
No. of polychromatic erythrocytes (PCE) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	Not reported

3. **Details of slide preparation:** Immediately after CO_2 asphyxiation, the marrow from both femurs of each animal was aspirated into a syringe containing fetal bovine serum (FBS) and transferred to a centrifuge tube. Erythrocytes were collected by centrifugation. The supernatant was removed, the cells were resuspended by aspiration with a capillary pipette, and smears were prepared on clean microscope slides. The slides (2/animal) were air-dried and fixed with methanol. Bone marrow smears were stained in May-Gruenwald-Giemsa and permanently mounted. Slides were coded prior to evaluation and were scored 'blind'. Two thousand polychromatic erythrocytes (PCE)/animal were examined for the presence of micronuclei. To assess cytotoxicity, the ratio of polychromatic erythrocytes to normochromatic erythrocytes (PCE:NCE) was determined by examining a total of 1000 cells (PCE + NCE).

4. Evaluation criteria

a. **Assay validity:** The assay was considered valid if the following criteria were met:
- The mean mouse polychromatic erythrocyte (MPCE) frequency in the vehicle controls was within the historical control range, and
- The positive control induced a statistically significant ($p \leq 0.05$) increase in MPCEs

b. **Positive result:** The test article was considered to be positive for mutagenicity if the group mean MPCEs was statistically significantly increased at either sampling time and exceeded the historical control range.

5. **Statistical methods:** The MPCE data were analyzed using the Kastenbaum-Bowman tables which are based on the binomial distribution. All analyses were performed separately for each sex and sampling time, and significance was denoted at $p<0.05$.

II. REPORTED RESULTS: The dose formulations were analyzed for homogeneity and actual concentrations using HPLC. The dose formulations were homogeneous (RSD = 5.13-6.04%) and the actual concentrations were within 90.7-99.0% of nominal.

A. PRELIMINARY TOXICITY ASSAY: In the range-finding study, no mortality or clinical signs of toxicity were observed at any dose in either sex. Therefore the limit dose (2000 mg/kg) was selected for the micronucleus assay.

B. MICRONUCLEUS ASSAY: The results of the micronucleus assay were summarized in Table 8.4 on page 26 of the study report, which is included as an Attachment to this DER.

No clinical signs of toxicity or mortality were observed in either sex at any time point. No significant decrease in polychromatic erythrocyte to normochromatic erythrocyte ratio

(PCE:NCE) was observed, indicating that the test material was not toxic to the bone marrow. No significant increases in the MPCE frequency compared to controls were observed in any treatment group at either 24 or 48 hours post-dosing. The positive control (cyclophosphamide) induced increases (p#0.05) in MPCEs compared to the concurrent vehicle controls at 24 hours post-dosing.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** The investigators concluded that DPX-MAT28 did not induce micronuclei in bone marrow cells of mice at up to 2000 mg/kg (limit dose).

B. **REVIEWER COMMENTS:** No mortality or clinical signs of toxicity were observed at any dose or time point. No significant decrease in PCE:NCE ratio was observed, indicating that the test material was not toxic to the bone marrow. No treatment-related increases in the MPCE frequency were observed in any treatment group when compared to controls. The positive control (cyclophosphamide) induced the appropriate response.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

C. **STUDY DEFICIENCIES:** None

ATTACHMENT

The following attachment contains Table 8.4 from page 26 of MRID 47560022.

Table 8.4: Summary of Bone Marrow Micronucleus Analysis Following a Single Oral Dose of DPX-MAT28 in Mice

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Animals	**PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of *** MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
Sterile water	M	24	5	0.465 ± 0.04	---	0.1 ± 0.22	1 / 10000
	F	24	5	0.447 ± 0.05	---	0.2 ± 0.27	2 / 10000
DPX-MAT28							
500 mg/kg	M	24	5	0.375 ± 0.05	-19	0.4 ± 0.22	4 / 10000
	F	24	5	0.398 ± 0.05	-11	0.3 ± 0.45	3 / 10000
1000 mg/kg	M	24	5	0.483 ± 0.05	4	0.3 ± 0.27	3 / 10000
	F	24	5	0.472 ± 0.05	6	0.3 ± 0.27	3 / 10000
2000 mg/kg	M	24	5	0.464 ± 0.05	0	0.0 ± 0.00	0 / 10000
	F	24	5	0.457 ± 0.06	2	0.3 ± 0.27	3 / 10000
Cyclophosphamide							
50 mg/kg	M	24	5	0.380 ± 0.09	-18	8.9 ± 1.47	*89 / 10000
	F	24	5	0.396 ± 0.04	-11	9.6 ± 1.67	*96 / 10000
Sterile water	M	48	5	0.448 ± 0.04	---	0.0 ± 0.00	0 / 10000
	F	48	5	0.434 ± 0.05	---	0.6 ± 0.22	6 / 10000
DPX-MAT28							
2000 mg/kg	M	48	5	0.495 ± 0.03	10	0.3 ± 0.45	3 / 10000
	F	48	5	0.469 ± 0.02	8	0.3 ± 0.45	3 / 10000

*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables).
**PCEs: Polychromatic Erythrocytes.
***MPCEs: Micronucleated Polychromatic Erythrocytes.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.6200a [§81-8a]; Neurotoxicity Screening Battery in Rats

Work Assignment No. 6-1-211 B (MRID 47725702)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
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Prepared by
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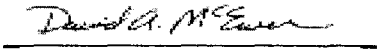
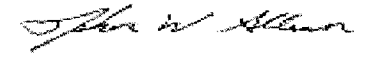
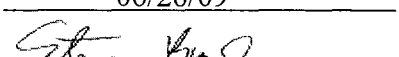
Primary Reviewer: David A. McEwen, B.S.	Signature:  Date: 06/26/09
Secondary Reviewer: John W. Allran, M.S.	Signature:  Date: 06/26/09
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature:  Date: 06/26/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature:  Date: 06/26/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:** 10/14/2009
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10-14-2009
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a [§81-8a]; OECD 424.

PC CODE: 288008 **DP BARCODE:** D361080
TXR #: 0055188

TEST MATERIAL (PURITY): DPX-MAT28 (92.2% a.i.)

SYNONYMS: Aminocyclopyrachlor; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Barnett Jr., J.F. (2009) Oral (gavage) acute neurotoxicity study of DPX-MAT28-009 in rats. Charles River Laboratories Preclinical Services, Horsham, PA. Laboratory Project ID: DuPont-22792, Project No. AUV00038, January 23, 2009. MRID 47725702. Unpublished.

SPONSOR: DuPont Haskell Global Centers for Health and Environmental Sciences, 1090 Elkton Road, Newark, DE

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 47725702), DPX-MAT28 (92.2% a.i.; Lot # 009) in 0.5% aqueous methylcellulose was administered once via gavage (5 mL/kg) to 10 Sprague-Dawley rats/sex/group at dose levels of 0, 200, 1000, or 2000 mg/kg (limit dose). Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all rats at pre-dosing and Days 1 (approximately 2 hours post-dosing; estimated time of peak effect), 8, and 15. At study termination, 5 rats/sex/group were anesthetized and perfused *in situ* for neuropathological examination. The tissues from the perfused animals in the control and 2000 mg/kg groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Acceptable positive control data were provided.

There were no treatment-related effects on mortality, clinical signs of toxicity, body weight, food consumption, brain weight, body weight gain, gross pathology, or neuropathology. FOB and motor activity testing revealed no treatment-related effects.

There were no neurological effects observed at any dose in either sex.

The LOAEL was not observed. The NOAEL is 2000 mg/kg (limit dose).

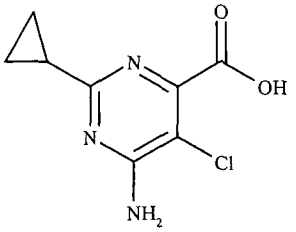
The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.6200a) for an acute neurotoxicity study in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** DPX-MAT28

Description:	White powder
Lot #:	009
Purity:	92.2% a.i.
Stability:	The test material was shown to be stable in the vehicle for up to 5 hours.
CAS # of TGAI:	858956-08-8
Structure:	

2. **Vehicle:** 0.5% Aqueous methylcellulose

3. **Test animals**

Species:	Rat	
Strain:	Crl:CD(SD)	
Age/mean weight at Day 1:	Approximately 6 weeks/ 267.4-273.3 g males and 199.8-205.4 g females	
Source:	Charles River Laboratories, Inc. (Raleigh, NC)	
Housing:	Individually in stainless steel, wire-bottomed cages	
Diet:	Certified Rodent Diet® #5002 (PMI Nutrition International, St. Louis, MO); *ad libitum*	
Water:	Reverse osmosis treated tap water with chlorine added as a bacteriostat; *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	At least 10/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	3 days	

B. STUDY DESIGN

1. **In-life dates:** Start: May 19, 2008 End: June 6, 2008

2. **Animal assignment and treatment:** Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. The initial weight variation of the rats used on study did not exceed ±20% of the mean body weight of each sex. The animals were subdivided into 4 replicate groups to accommodate the neurobehavioral testing schedule.

TABLE 1. Study design [a]				
Experimental parameter	Dose (mg/kg)			
	0	200	1000	2000
Total number of animals/sex/group	10/sex	10/sex	10/sex	10/sex
Behavioral testing (FOB, Motor activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	5/sex	5/sex	5/sex

a Data were extracted from pages 25 and 28 of the study report.

3. **Dose rationale:** The doses for the current study were selected based on the results of previous studies (study numbers not reported). It was stated that because there have been no previous adverse clinical signs observed after administration of the test material, the limit dose (2000 mg/kg) was selected as the high dose in the current study. In a previous acute oral toxicity study (study number not reported), following a dose of 5000 mg/kg diarrhea was observed on Day 2. Therefore, the FOB and motor activity evaluations in the current study were performed at 2 hours post-dosing, a time at which the test material was expected to have been absorbed.

4. **Test Substance preparation, administration, and analysis:** Test suspensions were prepared once by mixing the appropriate amount to the test material with 0.5% (w/v) methylcellulose and stored at room temperature until needed. Dose formulations were administered once via gavage at a volume of 5 mL/kg. Homogeneity (top, middle, bottom) and concentration analyses were performed on samples from all doses collected prior to dosing on Day 1. The stability of the 40 and 400 mg/mL formulations was confirmed for 5 hours at room temperature, and dose formulations were used within 5 hours of preparation.

Results

Homogeneity analysis (range as % relative standard deviation): 2-4%

Stability analysis (range as % nominal after 5 hour storage at room temperature): 104-105%

Concentration analysis (range as mean % of nominal): 105.5-113.0%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** The data were analyzed using the following statistical methods.

Parameter	Statistical Procedures
FOB parameters using interval scales (such as grip strength tests and landing foot splay test), body weight data, food consumption, motor activity	Bartlett's Test of Homogeneity of Variances was performed. If the result was not significant ($p>0.001$), then ANOVA was performed. When ANOVA was significant ($p\leq 0.05$), Dunnett's test was conducted. If Bartlett's test revealed heterogeneity of variances, the Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq 0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Motor activity	Repeated measures ANOVA
FOB having graded or count scores	The Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq 0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Clinical observation incidence data, FOB descriptive and quantal data	Contingency tables using the Variance Test for Homogeneity of the Binomial Distribution were used.
Neuropathology findings	The Fisher's Exact Test was conducted.

Statistical significance was denoted at $p<0.05$ or $p<0.01$. The reviewers consider the analyses used to be appropriate.

C. METHODS / OBSERVATIONS

1. **Mortality and clinical observations:** Animals were observed twice daily for mortality and moribundity throughout the study. All animals were evaluated for clinical signs of toxicity once daily as part of the FOB (pre-exposure, prior to test material administration on Day 1, and daily thereafter).

2. **Body weight:** Animals were weighed daily, including the days that the FOB was performed (pre-exposure and Days 1, 8, and 15), and at termination.

3. **Food consumption:** Food consumption was recorded daily and reported as absolute (g/animal/day) and relative to body weight (g/kg/day) values.

4. **Cholinesterase determination:** Cholinesterase activity was not determined.

5. **Ophthalmoscopic examinations:** Ophthalmoscopic exams were not performed.

6. Neurobehavioral assessment

a. Functional observational battery (FOB): The FOB was conducted prior to initiation of dosing and on Days 1 (approximately 2 hours post-dosing; estimated time of peak effect), 8, and 15. The technician conducting the evaluations was unaware of the dose group assignment of the animals. The scoring criteria and the duration of the open-field evaluations were not provided. The following CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*	X	Mobility
	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
	Feces consistency	X	Respiratory rate+	X	Urination / defecation*
			Red/crusty deposits*		Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*	X	Gait score*
X	Touch response+		Muscle tone*	X	Bizarre / stereotypic behavior*
X	Startle response*	X	Vocalization		Backing
X	Pain response*				Time to first step
X	Pupil response*				
	Surface righting response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
X	Visual placing response				Rotarod performance

*Required parameters; +Recommended parameters

b. Locomotor activity: Locomotor activity was evaluated following the FOB (prior to initiation of dosing and on Days 1 [approximately 2 hours post-dosing; estimated time of peak effect], 8, and 15). The movements of each rat were monitored by a passive infrared sensor mounted outside a stainless steel, wire-bottom cage during a 1 hour period, with the number of movements and time spent in movement tabulated at 5 minute intervals. Groups were counterbalanced across test sessions and cages.

7. **Sacrifice and pathology:** On Day 16, all animals were sacrificed by an intravenous injection of sodium heparin, and then exsanguinated and perfused *in situ* with neutral buffered 10% formalin, and a gross necropsy was performed. The calvarium was removed, and the head was immersed in the fixative. After 24 hours, the brain was excised and weighed. In rats not selected for neurohistological examination, the vertebral column was cut into segments, and the hindlimbs were removed and dissected to expose the peripheral nerves. These tissues were immersed in the fixative and, along with the head and brain, retained for possible evaluation. In the animals selected for evaluation of neuropathological effects (5 rats/sex/dose), tissues were further dissected to allow the evaluation of the Gasserian ganglion, spinal cord, peripheral nerves, skeletal muscles, brain, and eye as detailed in the table below. The following CHECKED (X) tissues from the control and 2000 mg/kg groups were evaluated microscopically by Charles River Laboratories Pathology Associates (Frederick, MD).

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Center of cerebrum	X	Sciatic notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve (proximal and distal)
	SPINAL CORD	X	Peroneal nerve (fibular)
X	Cervical swelling	X	Cervical dorsal root ganglion
X	Thoracic swelling	X	Cervical dorsal root fibers
X	Lumbar swelling	X	Cervical ventral root fibers
	OTHER		Thoracic dorsal root ganglion
X	Gasserian ganglion		Thoracic dorsal root fibers
X	Trigeminal nerves		Thoracic ventral root fibers
X	Optic nerve	X	Lumbar dorsal root ganglion
X	Eyes	X	Lumbar dorsal root fibers
X	Gastrocnemius muscle	X	Lumbar ventral root fibers

The central nervous system tissues were trimmed, processed, embedded in paraffin, and sectioned (5 µm). The peripheral nerves were embedded in plastic, and sectioned (2 µm). Sections were stained with hematoxylin and eosin (H&E), luxol fast blue/cresyl violet or toluidine blue, Bielschowsky's technique, or by other methods when appropriate. Longitudinal and transverse sections were made of the cervical, thoracic, and lumbar spinal cord; and the sciatic, tibial, fibular (longitudinal only), and sural nerves.

8. **Positive controls:** Summary data from two studies (Argus Study Nos. 012-058 and 012-104; performed in 1996 and 2002, respectively) were provided that generated positive control data and validated the procedures and observers of the performing lab to conduct the FOB and to assess motor activity effects. In the FOB portion of these studies, exposure to **IDPN** (250 mg/kg; 4-5 daily i.p. doses at 1 mL/kg) induced the following effects at 3 days post-dosing:

(i) stereotypic behavior; (ii) slight ataxia; (iii) increased ($p<0.05$) gait abnormality; (iv) impaired air-righting reflex; (v) decreased ($p<0.05$) hindlimb grip strength; and (vi) decreased ($p<0.01$) body weight (males). At 10 days post-dosing, additional signs observed included increased ($p<0.01$) foot splay in the males and abnormal respiratory rate in both sexes. The following effects were noted at 60 minutes post-dosing with **chlorpromazine** (6 mg/kg; single i.p. dose at 1 mL/kg): (i) unusual posture; (ii) decreased mobility in the open-field; (iii) palpebral closure (eyes half-closed); (iv) lacrimation; (v) impaired air-righting reflex; and (vi) decreased ($p<0.01$) body temperature (males). Exposure to **d-amphetamine** (4 mg/kg; single i.p. dose at 1 mL/kg) induced stereotypic behavior and piloerection at 30 minutes post-dosing. **Carbaryl** (100 mg/kg; single oral dose at 4 mL/kg) induced the following effects at 30 minutes post-dosing: (i) unusual behavior; (ii) whole body tremors; (iii) limb twitches; (iv) unusual posture; (v) slight to moderately impaired gait (ataxic, limbs splayed/dragging, or tip-toe); (vi) lacrimation; (vii) salivation; (viii) decreased pupil response; (ix) decreased ($p<0.01$) body temperature; and (x) increased ($p<0.05$) foot splay (females). Exposure to **DDT** (75 mg/kg; single oral dose at 2 mL/kg) induced the following effects at 6 hours post-dosing: (i) unusual behavior; (ii) limb twitches; (iii) whole body tremors; (iv) abnormal respiration; and (v) impaired air-righting reflex. In the motor activity test, exposure to **acrylamide** (45 mg/kg; 10 daily i.p. doses at 1 mL/kg) induced decreases ($p<0.05$) in the number of moves and time spent in motion in both sexes, and **d-amphetamine** (0.75 mg/kg; 3 i.p. doses at 1 mL/kg) induced increases ($p<0.05$) in the number of moves and time spent in motion in both sexes. No positive control data that demonstrate the ability of the performing laboratory to identify neuropathological lesions were provided. However, as the tissue samples were sent to an independent pathology laboratory for evaluation, this lack of data is not considered to be a deficiency.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All animals survived to scheduled sacrifice.

2. **Clinical signs:** No treatment-related clinical signs of toxicity were observed at any dose in either sex.

B. BODY WEIGHT AND BODY WEIGHT GAIN:

No statistically significant differences in body weight and body weight gain were observed in any treated groups compared to controls (Table 2). In the 2000 mg/kg males, overall (Days 1-16) body weight gain was decreased by 17% compared to controls.

TABLE 2. Mean (±SD) body weight and body weight gain (g) in rats exposed to DPX-MAT28 once via gavage. [a]

Days	Dose (mg/kg)			
	0	200	100	2000
Males				
1	273.3±17.2	267.9±16.9	267.4±13.1	271.6±19.3
16	370.2±42.8	350.9±38.6	356.5±29.9	351.6±44.6
Overall (Days 1-16) gain	96.9±27.4	83.0±22.9	89.1±19.5	80.0±25.9 (↓17)
Females				
1	199.8±13.4	205.4±11.2	202.0±10.7	203.0±8.7
16	239.2±16.6	246.6±19.5	242.8±19.6	246.5±14.2
Overall (Days 1-16) gain	39.4±10.1	41.2±11.0	40.8±11.8	43.5±7.3

a Data were extracted from Tables 3-6 on pages 60-63 of the study report; n=10. Percent difference from controls (calculated by reviewers) is presented parenthetically.

C. **FOOD CONSUMPTION:** At 1000 and 2000 mg/kg, both absolute (↓12-17%) and relative (↓10-17%) food consumption were decreased compared to controls in both sexes on Days 1-2 (Table 3a and 3b). The decreases in relative food consumption attained statistical significance in the 1000 mg/kg females and in both sexes at 2000 mg/kg.

TABLE 3a. Mean (±SD) absolute (g/animal/day) food consumption in rats exposed to DPX-MAT28 once via gavage. [a]

Interval (Days)	Dose (mg/kg)			
	0	200	1000	2000
Males				
1-2	24.6±3.9	23.2±3.7	21.7±2.8 (↓12)	20.4±3.9 (↓17)
2-3	26.9±2.8	25.7±3.0	25.5±3.2	29.6±11.0
1-8	24.6±2.6	23.5±2.5	23.4±1.6	23.7±3.2
8-16	25.9±4.0	23.4±2.4	23.6±1.9	23.4±3.4
Overall (Days 1-16)	25.2±3.3	23.4±2.4	23.5±1.7	23.6±3.0
Females				
1-2	15.2±2.3	17.2±3.2	13.1±1.7 (↓14)	13.2±1.7 (↓13)
2-3	16.9±3.4	16.5±3.6	16.7±3.7	16.6±3.3
1-8	17.5±1.4[b]	17.9±1.7	16.6±1.4	16.8±1.5
Overall (Days 1-16)	18.1±1.4	18.9±1.3	17.6±0.8	18.1±1.4

a Data were extracted from Tables 7 and 9 on pages 64 and 66 of the study report; n=10. Percent difference from controls (calculated by reviewers) is presented parenthetically.

b n=9, excludes values that appeared incorrectly recorded or were associated with spillage.

TABLE 3b. Mean (±SD) relative (g/kg/day) food consumption in rats exposed to DPX-MAT28 once via gavage. [a]

Interval (Days)	Dose (mg/kg)			
	0	200	1000	2000
Males				
1-2	89.0±11.3	85.5±10.8	80.1±9.1 (↓10)	74.1±12.5** (↓17)
2-3	95.9±8.9	93.6±8.6	92.6±11.2	105.8±39.7
1-8	82.9±4.6	81.4±3.8	80.8±2.8	81.3±8.0
8-16	75.1±6.1	71.1±4.0	70.6±1.4	70.6±3.9
Overall (Days 1-16)	78.5±4.8	75.6±3.3	75.2±1.7	75.4±4.4
Females				
1-2	75.5±10.9	82.9±14.1	64.8±8.5* (↓14)	64.3±8.5* (↓15)
2-3	82.7±15.6	78.2±14.9	80.8±15.2	79.3±14.3
1-8	83.4±6.5[b]	82.8±5.9	78.4±4.2	78.6±4.8
Overall (Days 1-16)	82.4±6.1	83.5±3.1	79.3±3.5	80.7±3.4

a Data were extracted from Tables 8 and 10 on pages 65 and 67 of the study report; n=10. Percent difference from controls (calculated by reviewers) is presented parenthetically.
b n=9, excludes values that appeared incorrectly recorded or were associated with spillage.
* Statistically significantly different from the control at $p<0.05$
** Statistically significantly different from the control at $p<0.01$

D. CHOLINESTERASE ACTIVITY: Cholinesterase activity was not evaluated.

E. OPHTHALMOSCOPIC EXAMINATIONS: Ophthalmoscopic exams were not performed

F. NEUROBEHAVIORAL RESULTS

1. **FOB findings:** No treatment-related effects were observed in any FOB parameter in either sex. In the 2000 mg/kg females, mean maximum hindlimb grip strength was increased ($p<0.05$) by 26% compared to controls on Day 8. However, this increase was due to an extreme value (592.5 g) from one rat (#18571). When this value was excluded, the mean value (391.7 g) was similar to the controls at this time point (328.5 g). As this finding was also transient, it was not considered to be related to treatment. All other statistically significant differences noted were not dose-dependent.

2. **Motor activity:** No statistically significant differences in number of movements or time spent in movement were observed for the total session or at any interval (Table 4). Habituation was unaffected by treatment.

TABLE 4. Mean (±SD) total session locomotor activity in rats exposed to DPX-MAT28 once via gavage. [a]

Observation	Dose (mg/kg)			
	0	200	1000	2000
Males				
Number of movements				
Pretest	680.6±144.7	587.7±157.0	580.2±184.5	580.4±140.4
Day 1 (2 hrs post-dosing)	360.5±78.7	334.1±98.4	429.7±180.0	353.3±78.5
Day 8	539.9±196.1	428.5±151.7	620.9±202.9	530.3±209.5
Day 15	639.5±216.8	683.6±144.2	708.9±162.9	731.5±188.2
Time spent in movement (sec)				
Pretest	1363.1±326.2	1074.9±289.6	1186.5±532.0	1106.4±206.8
Day 1 (2 hrs post-dosing)	759.2±213.1	640.9±232.3	839.0±332.1	680.3±127.6
Day 8	1161.1±443.4	851.9±306.3	1273.3±422.4	1056.4±391.2
Day 15	1462.4±608.6	1406.0±410.3	1525.6±510.9	1389.1±343.6
Females				
Number of movements				
Pretest	700.8±78.1	675.7±146.7	621.6±189.4	671.7±143.1
Day 1 (2 hrs post-dosing)	496.6±91.6	550.9±170.7	473.7±104.4	469.9±172.1
Day 8	600.2±176.5	564.4±219.3	598.0±80.1	546.6±103.1
Day 15	644.5±191.6	588.0±129.7	596.4±132.5	620.4±126.3
Time spent in movement (sec)				
Pretest	1354.0±310.5	1311.1±267.8	1200.2±429.2	1311.6±348.7
Day 1 (2 hrs post-dosing)	885.2±208.4	1001.4±255.6	849.8±250.9	990.6±464.6
Day 8	1218.0±387.1	1109.5±456.8	1259.4±212.7	1030.7±317.7
Day 15	1259.9±464.4	1146.3±365.9	1145.5±266.2	1173.9±323.1

a Data were extracted from Tables 13 and 14 on pages 116-131 of the study report; n=10.

G. SACRIFICE AND PATHOLOGY

1. **Gross pathology:** No treatment-related gross lesions were noted in any animal.

2. **Brain weight:** No treatment-related effects on brain weight were observed at any dose in either sex (Table 5). The decrease ($p<0.05$) in absolute brain weight noted in the 200 mg/kg males (↓4%) was minor and not dose-dependent.

TABLE 5. Mean (±SD) absolute (g) and relative brain weights (%) in rats exposed to DPX-MAT28 once via gavage. [a]

Parameter	Dose (mg/kg)			
	0	200	1000	2000
Males				
Terminal Body (g)	370.2±42.8	350.9±38.6	356.5±29.9	351.6±44.6
Absolute Brain (g)	2.224±0.059	2.139±0.054* (↓4)	2.238±0.099	2.184±0.100
Relative (to body) Brain (%)	0.606±0.076	0.616±0.058	0.629±0.039	0.626±0.055
Female				
Terminal Body (g)	239.2±16.6	246.6±19.5	242.8±19.6	246.5±14.2
Absolute Brain (g)	2.093±0.111	2.065±0.062	2.098±0.075	2.097±0.094
Relative (to body) Brain (%)	0.877±0.051	0.842±0.072	0.868±0.066	0.852±0.051

a Data were extracted from Tables 17 and 18 on pages 134-135 of study report; n=10. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Statistically significantly different from the control at $p<0.05$

3. **Neuropathology:** No treatment-related neuropathological lesions were observed at any dose in either sex. Lesions noted included minimal nerve fiber degeneration in the trapezoid body of the brainstem, sections of spinal cord, peripheral nerves, and spinal nerve roots, and vacuolation of neurons in either the Gasserian ganglia or dorsal root ganglia. These lesions were minimal to mild in severity, were observed with equal or greater frequency in the control animals, and are commonly observed in rats of this strain and age.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** The investigators concluded that the NOAELs for general toxicity and neurotoxicity were both 2000 mg/kg.

B. **REVIEWER COMMENTS:** The test article is listed as DPX-MAT28-009 in the title of the study report. The Certificate of Analysis clearly states that the lot number is 009. So, DPX-MAT28-009 is the same as DPX-MAT28.

There were no treatment-related effects on mortality, clinical signs of toxicity, body weight, brain weight, gross pathology, or neuropathology. FOB and motor activity testing revealed no treatment-related effects.

At 2000 mg/kg, overall (Days 1-16) body weight gain was decreased by 17% in the males compared to controls. Additionally at 1000 and 2000 mg/kg, both absolute and relative food consumption were decreased by 10-17% compared to controls in both sexes on Days 1-2. The decreases in relative food consumption attained statistical significance in the 1000 mg/kg females and in both sexes at 2000 mg/kg. However, the decreases in food consumption were transient, and overall (Days 1-16) food consumption values for both absolute and relative

food consumption were similar to controls in both sexes. Furthermore, the decrease in body weight gain in the 2000 mg/kg males was not statistically significant. Therefore, these findings were not considered to be biologically relevant.

There were no neurological effects observed at any dose in either sex.

The LOAEL was not observed. The NOAEL is 2000 mg/kg (limit dose).

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.6200a) for an acute neurotoxicity study in rats.

C. **STUDY DEFICIENCIES:** None

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.7485 [§85-1]; Metabolism Study in Rats

Work Assignment No. 5-01-209 N (MRID 47560023)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Primary Reviewer: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 3/30/09
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Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 3/30/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Steven Brecher Date: 3/30/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** 10-14-2009
Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Risk Assessment Branch III, Health Effects Division (7509P) **Date:** 10/15/2009

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 [§85-1]; OECD 417.

PC CODE: 288008 **DP BARCODE:** D361080
TXR#: 0055188

TEST MATERIAL (RADIOCHEMICAL PURITY): Aminocyclopyrachlor (99.5%)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Himmelstein, M.W. (2008) ^{14}C-DPX-MAT28: plasma pharmacokinetics and pilot material balance in male and female rats. E.I. du Pont de Nemours and Company, Dupont Haskell Global Centers for Health & Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-22033, August 18, 2008. MRID 47560023. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY In this metabolism study (MRID 47560023), [pyramidine-2-^{14}C]-DPX-MAT28 (aminocyclopyrachlor; Lot No. 01; radiochemical purity 99.5%) in 0.5% methylcellulose (4 ml/kg) was administered by a single oral gavage to groups of Sprague Dawley rats for the following studies: (i) a pilot mass balance study conducted on one rat/sex dosed at 25 mg/kg; (ii) a blood pharmacokinetic study conducted on four rats/sex given either a 25 or 500 mg/kg dose; and (iii) a plasma metabolite study conducted on three rats/sex dosed at 500 mg/kg. Metabolite profiles were also determined for urine and feces collected in the pilot mass balance study.

DPX-MAT28 was absorbed with peak concentrations occurring in plasma and RBCs at 0.3-1.0 h post-dosing. The AUC for DPX-MAT28 was 7.0-9.0 μg*h/g at 25 mg/kg and 150.8-168.4 μg*h/g at 500 mg/kg, a difference of approximately 19 to 21-fold, which scaled with the 20-fold difference in dose. The majority of the whole blood radiolabel was found in the plasma (60%), with about 40% in RBCs, Total recoveries of the administered dose (AD) were 69.9% for the male (which is low) and 107.9% for the female. DPX-MAT28 was rapidly eliminated after a single oral gavage dose, with mean elimination half-life values of 5.6-5.7 h for plasma at 25 or 500 mg/kg. The majority of radioactivity was recovered 0-24 h post-dosing, with 35.8-53.71% eliminated in the urine of males and females and 31.4-48.0% eliminated in the feces of males and

females No radioactivity was detected in the expired air as exhaled volatiles or CO_2 for DPX-MAT28. Cage wash accounted for a small portion (0.36-1.21% AD) of radioactivity, and a minor amount of radioactivity was detected in the residual feed. At 168 h post-dosing, the residual carcass of the male accounted for 0.04% AD; radioactivity was below the limit of detection in the collected tissues from both sexes and in the female residual carcass.

Plasma, urine, and feces were examined for metabolites at 30 minutes, 24 hours, and 24 hours, respectively. Only a single peak was observed in each matrix, and was identified as unmetabolized parent. In a concurrently reviewed subchronic oral toxicity study in rats (MRID 47573403), a metabolite (IN-LTX69) was quantified in plasma; however, this metabolite was not detected in the present study following a single dose of the test compound. Control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled DPX-MAT28 dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days; no noticeable differences were observed, indicating that the test compound was stable in the plasma, urine, and feces, and in the prepared sample extracts.

This metabolism study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OECD 417] in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound

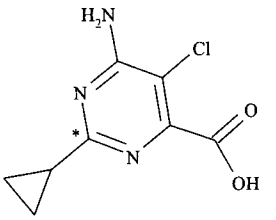
Radiolabeled test material: [Pyrimidine-2-^{14}C]-DPX-MAT28

Radiochemical purity: 99.5% (HPLC)

Specific activity: 42.42 µCi/mg

Lot number: 01

Structure



* = position of the [^{14}C]-label

Non-Radiolabeled test material:

Description: Solid

Lot number: DPX-MAT28-012

Purity: 99.7%

CAS # of TGAI: 858956-08-8

2. Vehicle: 0.5% methylcellulose

3. Test animals

Species: Rat

Strain: Sprague Dawley (Crl:CD[SD])

Age/weight at study initiation: At least 8 weeks; 227.5-278.8 g males, 165.1-218.0 g females

Source: Charles River Laboratories, Inc. (Raleigh, NC)

Housing: Individually in glass metabolism cages

Diet: Certified Rodent LabDiet® 5002 (PMI® Nutrition International, LLC, St. Louis, MO), *ad libitum*, except prior to dosing (period not specified) and for 2 h post-dosing.

Water: Tap water, *ad libitum*.

Environmental conditions:
- **Temperature:** 18-26°C
- **Humidity:** 30-70%
- **Air changes:** Not provided
- **Photoperiod:** 12 h light/12 h dark

Acclimation period: At least 6 days (cannulated rats at least 3 days)

4. Preparation of dosing solutions:

Dose levels of 25 and 500 mg/kg were administered by single oral gavage. Dosing solutions were prepared (time prior to dosing not specified) by weighing appropriate amounts of radiolabeled test compound, non-radiolabeled test compound, and vehicle into a vial and mixing to yield a clear solution or homogeneous suspension. After preparation, the dose suspensions were stored in a refrigerator. It was stated that prior to administration, samples of each suspension were analyzed for radioactive concentration by liquid scintillation counting (LSC), and for chemical concentration and radiochemical purity by HPLC. After dose administration, each suspension was reanalyzed for radiochemical purity to determine stability. However, concentration, radiochemical purity, and stability data were not provided; the Sponsor stated that mean dose administration data indicated that all animals received the target oral dose for each treatment group.

B. STUDY DESIGN AND METHODS

1. **Group arrangements:** Animals were assigned to the test groups noted in Table 1. Cage-side examinations to detect moribund or dead rats and abnormal behavior and/or appearance were conducted at least once daily throughout the study.

TABLE 1: Dose groups for [pyrimidine-2-^{14}C]-DPX-MAT28 metabolism studies [a]

Test group	Nominal dose (mg/kg)	Actual mean dose (mg/kg)	Animals /group	Comments
Pilot mass balance	25	25.1/25.0 (M/F)	1/sex	Urine, feces, cage rinse, residual food, and exhaled volatiles and CO_2 were collected at regular intervals. Blood, tissues, and residual carcass were collected at 168 h post-dosing. Metabolite profiles were determined for urine and feces.
Pharmacokinetics	25	25.0/25.3 (M/F)	4/sex[b]	Blood samples were collected from jugular vein cannulas at regular intervals up to 30 h post-dosing.
	500	482.5/481.8 (M/F)		
Metabolism	500	499.1/497.5 (M/F)	3/sex[c]	Blood was collected at 30 minutes post-dosing, and a metabolite profile was determined for plasma.

a Data were obtained from page 13 of the study report.
b An additional one rat/sex was dosed at 500 mg/kg (actual dose 474.1/478.3 mg/kg in M/F); blood was collected at 2 h post-dosing for methods development for the metabolite profiles.
c Control blood was collected from one rat/sex for comparative purposes.

2. **Dosing and sample collection:** Rats were dosed by oral gavage with dose amounts based on individual body weights immediately prior to dosing and a dosing volume of 4 mL/kg. The rats were fasted for an unspecified period of time prior to dosing. The target radioactive dose was 30 µCi/250 g animal. The actual specific activities of the dosing formulations were 5.10-6.55 µCi/mg for the 25 mg/kg formulations and 0.23-0.25 µCi/mg for the 500 mg/kg formulations. The method used to determine the actual administered dose was not provided; actual mean doses are presented in Table 1.

a. **Pilot mass balance study:** One rat/sex was dosed with [^{14}C]-DPX-MAT28 at 25 mg/kg. Urine and feces were collected separately into dry ice cooled receivers at 24 h intervals for a total of 168 h. Expired air was routed through traps containing ethylene glycol (for volatiles), 2N NaOH (for CO_2), and water (carryover) for sampling at 24 h intervals for a total of 48 h. Residual feed was collected for radioanalysis throughout the study. Cages were rinsed with water (times not specified), and the rinse was collected throughout the study into a common container as needed to keep the urine and feces separator functional. At the end of the experiment, cages were rinsed with detergent and water (50/50 v/v), water, and acetone into the same container used for the water rinses. Cages were also wipe-tested to determine the efficiency of the cage rinses.

At study termination (168 h post-dosing), the rats were killed by CO_2 asphyxiation followed by exsanguination. The following tissues were collected for radioanalysis.

Blood (plasma and RBC)[a]	Fat	Liver
Kidney	Muscle	Heart
Lung	Testes	Ovaries
Uterus	Bone/bone marrow[a]	Brain
Spleen	Adrenals	Pituitary
GI tract and contents[a]	Pancreas	Skin
Thyroid	Thymus	Bladder[b]

a Analyzed separately.
b Urine in the bladder at termination was aspirated and combined with the terminal urine sample.

Whole blood was centrifuged to separate plasma and red blood cells. After collection, tissue samples were stored at approximately -10°C until processing and analysis. The residual carcasses were homogenized and stored frozen until analysis.

b. **Pharmacokinetic study:** Four rats/sex/dose level were obtained from the vendor with surgically implanted jugular vein cannulas and were dosed with [^{14}C]-DPX-MAT28 at 25 or 500 mg/kg. Blood samples were collected from the cannulas prior to dosing and at 5, 15, and 30 minutes, and 1, 2, 4, 8, 12, 24, and 30 h post-dosing. Whole blood samples were kept on wet ice immediately following collection; plasma and RBC were separated by centrifugation. RBC were stored at 1-10°C. Plasma samples were held on wet ice or refrigerated if analysis was performed on the same day as collection; if not, plasma was stored frozen at <-10°C. All rats were killed by CO_2 asphyxiation followed by exsanguination and discarded after the final sample collection.

c. **Plasma metabolite profile study:** Three rats/sex were dosed with [^{14}C]-DPX-MAT28 at 500 mg/kg; an additional male and female were used for collection of control plasma. At 30 minutes post-dosing, all rats were killed by CO_2 asphyxiation followed by exsanguination. Whole blood was collected and separated into plasma and RBC. The plasma was stored frozen for metabolite analysis; the RBC were discarded.

d. **Sample analysis:** All samples were analyzed in triplicate, except blood samples from the pharmacokinetic study which were analyzed in duplicate. It was stated that if ongoing analysis of plasma indicated that the [^{14}C]-concentrations were approaching the limit of quantitation, a single maximum aliquot volume per time point was analyzed. The ethylene glycol, NaOH, and water trap contents, plasma, urine, and cage rinses were analyzed directly by LSC. Feces were homogenized in water and combusted; tissues, RBC, food residue, and residual carcasses were homogenized and combusted.

Radioactivity in all samples was quantitated by LSC. It was stated that samples were analyzed for 10 minutes, or until 160,000 disintegrations (0.5%; 2σ) were accumulated, whichever came first. The methods used for quench correction and background determination were not described, and the limit of detection was not defined.

e. **Metabolite characterizations:** Urine and fecal samples from the pilot mass balance study and plasma samples from the plasma metabolite profile study were examined for metabolites. An equal volume of methanol was added to each plasma sample (separately); the samples were then mixed, incubated overnight in a freezer to precipitate proteins, mixed again, and centrifuged. The supernatant was removed; an aliquot analyzed by LSC for

radioactivity, and the remainder was used for metabolite analysis. Urine samples (pre-dose and 0-24 h) were treated identically as the plasma samples, except the overnight incubation was omitted. Separate fecal homogenates (pre-dose and 0-24 h) were mixed homogeneously and a volume of 50:50 methanol:water (v/v) was added. Five ball bearings were added and the samples were further homogenized. The samples were then centrifuged, and the supernatants were decanted and filtered by syringe through 0.45 µm nylon membranes. An aliquot was analyzed by LSC, and the remainder was used for metabolite analysis.

Quantitation of metabolites and parent was performed using an HPLC coupled to an in-line radioactivity detector. Fractions were collected and appropriate fractions were analyzed for metabolite identification by LC/MS. Identifications were based on retention times and mass fragmentation patterns compared to reference standards. Additionally, control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to assess the stability of the test compound before and after sample preparation at 3 (feces), 10 (urine), and 14 (plasma) days.

3. **Statistics:** Statistical analyses were limited to calculations of mean and standard deviation. Pharmacokinetic parameters were calculated using WinNonlin Professional software (Pharsight Corporation, Mountain View, CA). $C_{max}/2$ (half-maximum concentration) was calculated as one-half C_{max}; $T_{max}/2$ (one-half time to maximum concentration) was estimated by interpolation.

II. RESULTS

A. **CLINICAL OBSERVATIONS:** No clinical signs of toxicity were noted during the cage side observations.

B. **PILOT MASS BALANCE STUDY:** Total recoveries were 69.9% of the administered dose (AD) for the male and 107.9% AD for the female (Table 2). The test compound was rapidly excreted, with the majority of radioactivity recovered 0-24 h post-dosing. Excretion in the urine and feces was approximately equal in magnitude; however, more radioactivity was recovered in the excreta of the female (55.8% AD in urine; 51.69% AD in feces) than the male (36.3% AD in urine; 32.1% AD in feces). No radioactivity was detected in the expired air during the first 48 h; therefore, collection was discontinued. Cage wash accounted for 1.21% and 0.36% AD in the males and females, respectively. A minor amount of radioactivity was detected in the residual feed (0.08-0.20% AD). At 168 h post-dosing, radioactivity was below the limit of detection in the collected tissues and in the female residual carcass; the residual carcass of the male accounted for 0.04% AD.

TABLE 2. Mean recovery of radioactivity (% administered dose) from rats administered a single 25 mg/kg dose of [^{14}C]-DPX-MAT28.[a]		
Sex	**Male**	**Female**
Urine		
0-24 h	35.89	53.71
24-48 h	0.28	1.51
48-72 h	0.09	0.53
72-168 h	0.04	0.01
Subtotal	36.30	55.76
Feces		
0-24 h	31.14	48.00
24-48 h	0.84	3.60
48-72	0.12	0.08
72-168 h	0.04	0.01
Subtotal	32.14	51.69
Expired air	<LOD	<LOD
Cage wash	1.21	0.36
Residual feed	0.20	0.08
Tissues	<LOD	<LOD
Carcass	0.04	<LOD
Recovery	**69.89**	**107.89**

a Data were obtained from Tables 2 and 3 on page 39 of the study report.
n=1 rat/sex
<LOD=less than limit of detection

C. **PHARMACOKINETIC STUDIES:** Plasma and RBC pharmacokinetic parameters are presented in Table 3. Absorption of the test compound was rapid, with radioactivity detected in both the plasma and RBC by 5 minutes post-dosing. The plasma elimination half-lives were essentially identical in the males (5.6 h) and females (5.7 h), and were unchanged by the 20-fold increase in dose. The mean peak concentrations in plasma of males and females were 3.8 and 5.0 µg equiv./g, respectively, at the 25 mg/kg dose level. These increased by 15-fold and 12-fold to 57.3 and 61.6 µg equiv./g for males and females at the 500 mg/kg dose level. The area under the plasma concentration-time curves ($AUC_{0\rightarrow\infty}$) values were 7.0 and 9.0 µg•h/g for males and females at 25 mg/kg, increasing by 22-fold and 19-fold to 150.8 and 168.4 µg•h/g for males and females at 500 mg/kg, respectively. The mean time to maximum plasma concentration were 0.5 h and 0.4 h for males and females, respectively, at 25 mg/kg, increasing slightly to 0.6 h and 1.0 h for males and females, respectively, at 500 mg/kg. In RBC, the mean peak concentrations were 1.3 and 2.0 µg equiv./g, respectively, at the 25 mg/kg dose level, increasing by 21-fold and 14-fold to 27.2 and 28.7 µg equiv./g for males and females at the 500 mg/kg dose level. The ratio of maximum concentration in RBC to plasma ranged from 0.33-0.48, indicating a limited potential for uptake and binding in the RBC.

TABLE 3. Mean (±SD) pharmacokinetic parameters for the plasma and RBC of rats following a single oral dose of [^{14}C]-DPX-MAT28.[a]

Parameter[b]	25 mg/kg		500 mg/kg	
	Male	Female	Male	Female
Plasma				
$t_{½}$ (h)	5.6±0.5	5.7±0.4	5.6±0.3	5.7±0.7
$AUC_{(0\rightarrow\infty)}$ (ug•h/g)	7.0±1.4	9.0±1.9	150.8±28.7	168.4±26.2
$AUC_{(0\rightarrow\infty)}$/dose (ug•h/g/mg)	0.3±0.1	0.4±0.1	0.3±0.1	0.3±0.1
C_{max} (µg equiv./g)	3.8±0.9	5.0±1.2	57.3±14.2	61.6±13.0
$C_{max}/2$ (µg equiv./g)	1.9	2.5	28.7	30.8
t_{Cmax} (h)	0.5±0.4	0.4±0.1	0.6±0.3	1.0±0.7
$t_{Cmax}/2$ (h)	1.4	1.2	2.0	2.3
RBC				
C_{max} (µg equiv./mL)	1.3±0.3	2.0±0.7	27.2±6.2	28.7±4.7
t_{Cmax} (h)	0.5±0.4	0.3±0.1	0.6±0.3	1.0±0.7
Ratio (C_{max} RBC/ C_{max} plasma)	0.33	0.40	0.48	0.47

a Data were obtained from Table 5 on page 41 of the study report. n=4 rats/sex

b Parameters: $t_{½}$ = terminal elimination half-life
AUC = area under concentration-time curve
C_{max} = maximum radioactivity concentration
t_{Cmax} = time to maximum concentration

D. METABOLITE CHARACTERIZATION STUDIES: The plasma samples collected 30 minutes after dosing at 500 mg/kg were initially analyzed for concentration of radioactivity; the mean concentrations were 250 and 296 µM for males and females, respectively. In plasma, urine, and feces, only a single peak identified as unmetabolized parent was observed in each matrix. Identification was confirmed by comparison of mass spectrograph data from the experimental matrices with data obtained from fortified control plasma, and fortified pre-dose urine and feces.

Control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days; no noticeable differences were observed, indicating that the test compound was stable in the plasma, urine, and feces, and in the prepared sample extracts.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: DPX-MAT28 administered by single oral gavage at 25 or 500 mg/kg was rapidly absorbed and excreted unchanged. The excretion in urine and feces accounted for approximately equal proportions of the total recovery within the first 24 hours after dosing. No radioactivity was detected in exhaled breath collected for 0-48 hours or in tissues collected at 168 hours, except for a minor percentage in the male rat carcass. Definitive pharmacokinetic data in plasma indicated rapid uptake and terminal elimination, with peak concentration and AUC values that were proportional with dose. The metabolite profile in plasma at 0.5 hours and in pilot urine and feces samples 0-24 hours after dosing were confirmed to contain only parent compound.

B. REVIEWER COMMENTS: The study report stated that no clinical signs of toxicity were noted during the cage side observations.

Absorption of the test compound was rapid, with radioactivity detected in both the plasma and RBC by 5 minutes post-dosing. The plasma elimination half-lives were essentially identical in the males and females, and were unchanged by the increase in dose. The mean peak concentrations in plasma increased by 12- to 15-fold from 25 mg/kg to 500 mg/kg. Similarly, the area under the plasma concentration-time curves ($AUC_{0\rightarrow\infty}$) at 25 mg/kg increased 19- to 22-fold at 500 mg/kg. The mean time to maximum plasma concentration increased slightly at 500 mg/kg compared to 25 mg/kg. In RBC, the mean peak concentrations increased by 21-fold and 14.4-fold from 25 mg/kg to 500 mg/kg. The ratio of maximum concentration in RBC to plasma indicated a limited potential for uptake and binding in the RBC.

Total recoveries were 69.9% AD for the male and 107.9% AD for the female. The recover in the male is unusually low. Data should be interpreted with caution, since only one animal was used. The test compound was rapidly excreted, with the majority of radioactivity recovered 0-24 h post-dosing. Excretion in the urine and feces was approximately equal in magnitude; however, more radioactivity was recovered in the excreta of the female than the male. No radioactivity was detected in the expired air during the first 48 h. Cage wash accounted for 1.21% and 0.36% AD in the males and females, respectively, and a minor amount of radioactivity was detected in the residual feed. At 168 h post-dosing, radioactivity was below the limit of detection in the collected tissues and in the female residual carcass; the residual carcass of the male accounted for 0.04% AD.

The plasma samples collected 30 minutes after dosing at 500 mg/kg were initially analyzed for concentration of radioactivity; the mean concentrations were 250 and 296 µM for males and females, respectively. In plasma, urine, and feces, only a single peak identified as unmetabolized parent was observed in each matrix. Identification was confirmed by comparison of mass spectrograph data from the experimental matrices with data obtained from fortified control plasma, and fortified pre-dose urine and feces. In a concurrently reviewed subchronic oral toxicity study in rats (MRID 47573403), a metabolite (IN-LTX69) was quantified in plasma; however, this metabolite was not detected in the present study following a single dose of the test compound. Additionally, control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days; no noticeable differences were observed, indicating that the test compound was stable in the plasma, urine, and feces, and in the prepared sample extracts.

This metabolism study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OECD 417] in rats.

C. **STUDY DEFICIENCIES:** The following deficiencies were observed that do not affect the acceptability of the study:

- Concentration, radiochemical purity, homogeneity and stability data for the dosing solutions were not provided. However, the actual mean doses presented were sufficiently close to the nominal doses to assure the reviewers that the concentration and homogeneity of the dosing solutions were within acceptable limits. Additionally, control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days; no noticeable differences were observed, indicating that the test compound was stable in the plasma, urine, and feces, and in the prepared sample extracts.
- The methods used for quench correction and background determination were not described, the limit of detection was not defined, and oxidizer efficiency was not provided.
- The pilot mass balance study was conducted on only one rat/sex, and recovery of the administered dose for the male (69.9%) was low.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR-METHYL (DPX-KJM44)

Study Type: OPPTS 870.7485 [§85-1]; Metabolism Study in Rats

Work Assignment No. 5-01-209 O (MRID 47560024)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 [§85-1]; OECD 417.

PC CODE: 288009 **DP BARCODE:** D361256
TXR#: 0055188

TEST MATERIAL (RADIOCHEMICAL PURITY): DPX-KJM44 (99.2%)

SYNONYMS: Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Himmelstein, M.W. (2008) ^{14}C-DPX-KJM44: plasma pharmacokinetics and pilot material balance in male and female rats. E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-22375, August 18, 2008. MRID 47560024. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In this metabolism study (MRID 47560024), [pyramidine-2-^{14}C]-DPX-KJM44 (Lot No. SEL/1539A; radiochemical purity 99.2%) in 4 ml/kg of 0.5% methylcellulose was administered by a single oral gavage to groups of Sprague Dawley rats for the following studies: (i) a pilot mass balance study conducted on one rat/sex dosed at 25 mg/kg; (ii) a blood pharmacokinetic study conducted on four rats/sex given either a 25 or 500 mg/kg dose; and (iii) a plasma metabolite study conducted on three rats/sex dosed at 500 mg/kg. Metabolite profiles were also determined for urine and feces collected in the pilot mass balance study.

The test compound was rapidly absorbed with peak concentrations occurring in plasma and RBC at 0.3-0.6 h post-dosing. The AUC for DPX-KJM44 was 14.5-14.8 µg*h/g at 25 mg/kg and 241.5-255.8 µg*h/g at 500 mg/kg, a difference of approximately 18-fold, which scaled with the 20-fold difference in dose. The majority of the whole blood radiolabel was found in the plasma (60%), with about 40% in RBCs. No differences were observed between the sexes.

Total recoveries of the administered dose (AD) were 89.2% for the male and 82.6% for the female. The plasma half-life for DPX-KJM44 was 8.7-13.3 h at 25 or 500 mg/kg . The majority

of radioactivity was recovered in the urine by 24 h post-dosing. (78.80-78.82%) for both males and females. A small amount was eliminated in the feces (1.72-4.57%) within 24 hours. No radioactivity was detected in the expired air as exhaled volatiles or CO_2 during the first 48. Cage wash accounted for a small portion (0.08-1.92% AD) of radioactivity, and a minor amount of radioactivity was detected in the residual feed. At 168 h post-dosing, radioactivity was below the limit of detection in the collected tissues except for the male gastrointestinal tract and contents (0.01% AD). Minor amounts of radioactivity were also detected in the residual carcass (0.12-0.14% AD).

Plasma, urine, and feces were examined for metabolites at 30 minutes, 24 hours, and 24 hours, respectively. Only a single metabolite, DPX-MAT28, (the free acid of DPXKJM44) was observed in each matrix. Identification was confirmed by comparison of mass spectrograph data from the experimental matrices with data obtained from fortified control plasma, and fortified pre-dose urine and feces. In a concurrently reviewed subchronic oral toxicity study in rats (MRID 47573403), a metabolite (IN-LTX69) was quantified in plasma; however, this metabolite was not detected in the present study following a single dose of the test compound.

Control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled DPX-KJM44 dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days. No noticeable differences were observed in the urine and feces or in the prepared sample extracts. However, the majority of DPX-KJM44 in plasma was metabolized to DPX-MAT28 when the sample was fortified and analyzed on the same day. Once the plasma was extracted, DPX-KJM44 appeared to be stable.

This metabolism study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OECD 417] in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

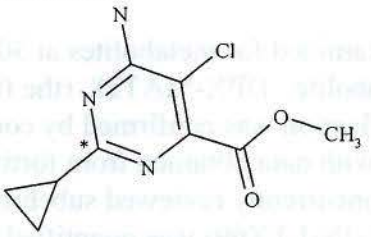
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound

Radiolabeled test material: [Pyrimidine-2-^{14}C]-DPX-KJM44

Radiochemical purity:	99.2% (HPLC)
Specific activity:	44.32 µCi/mg
Lot number:	SEL/1539A
Structure	



* = position of the [^{14}C]-label

Non-Radiolabeled test material:

Description:	Solid
Lot number:	031
Purity:	99.4%
CAS # of TGAI:	858954-83-3

2. Vehicle: 0.5% methylcellulose

3. Test animals

Species:	Rat
Strain:	Sprague Dawley (Crl:CD[SD])
Age/weight at study initiation:	At least 8 weeks; 205.5-339.2 g males, 166.2-226.1 g females
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Individually in glass metabolism cages
Diet:	Certified Rodent LabDiet® 5002 (PMI® Nutrition International, LLC, St. Louis, MO), *ad libitum*, except prior to dosing (period not specified). Food was returned 2 h post-dosing in the low dose groups and 4 h post-dosing in the high dose groups.
Water:	Tap water, *ad libitum*.
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** Not provided **Photoperiod:** 12 h light/12 h dark
Acclimation period:	At least 5 days (cannulated rats at least 3 days)

4. Preparation of dosing solutions:

Dose levels of 25 and 500 mg/kg were administered by single oral gavage. Dosing solutions were prepared (time prior to dosing not specified) by weighing appropriate amounts of radiolabeled test compound, non-radiolabeled test compound, and vehicle into a vial and mixing to yield a clear solution or homogeneous suspension. After preparation, the dose suspensions were stored in a refrigerator. It was stated that prior to administration, samples of each suspension were analyzed for radioactive concentration by liquid scintillation counting (LSC), and for chemical concentration and radiochemical purity by HPLC. After dose administration, each suspension was reanalyzed for radiochemical purity to determine stability. However, concentration, radiochemical purity, and stability data were not provided; the Sponsor stated that mean dose

administration data indicated that all animals received the target oral dose for each treatment group.

B. STUDY DESIGN AND METHODS

1. **Group arrangements:** Animals were assigned to the test groups noted in Table 1. Cage-side examinations to detect moribund or dead rats and abnormal behavior and/or appearance were conducted at least once daily throughout the study.

TABLE 1: Dose groups for [pyrimidine-2-^{14}C]-DPX-MAT28 metabolism studies [a]

Test group	Nominal dose (mg/kg)	Actual mean dose (mg/kg)	Animals /group	Comments
Pilot mass balance	25	24.8/24.8 (M/F)	1/sex	Urine, feces, cage rinse, residual food, and exhaled volatiles and CO_2 were collected at regular intervals. Blood, tissues, and residual carcass were collected at 168 h post-dosing. Metabolite profiles were determined for urine and feces.
Pharmacokinetics	25	24.7/25.2 (M/F)	4/sex[b]	Blood samples were collected from jugular vein cannulas at regular intervals up to 30 h post-dosing.
	500	464.8/482.1 (M/F)		
Metabolism	500	455.0/454.9 (M/F)	3/sex[c]	Blood was collected at 30 minutes post-dosing, and a metabolite profile was determined for plasma.

a Data were obtained from pages 13 and 37 of the study report.
b An additional one rat/sex was dosed at 500 mg/kg (actual dose 472.8/484.3 mg/kg in M/F); blood was collected at 2 h post-dosing for methods development for the metabolite profiles.
c Control blood was collected from one rat/sex for comparative purposes.

2. **Dosing and sample collection:** Rats were dosed by oral gavage with dose amounts based on individual body weights immediately prior to dosing and a dosing volume of 4 mL/kg. The rats were fasted for an unspecified period of time prior to dosing. The target radioactive dose was 30 µCi/250 g animal. The actual specific activities of the dosing formulations were 4.31-6.09 µCi/mg for the 25 mg/kg formulations and 0.22-0.24 µCi/mg for the 500 mg/kg formulations. The method used to determine the actual administered dose was not provided; actual mean doses are presented in Table 1.

a. **Pilot mass balance study:** One rat/sex was dosed with [^{14}C]-DPX-KJM44 at 25 mg/kg. Urine and feces were collected separately into dry ice cooled receivers at 24 h intervals for a total of 168 h. Expired air was routed through traps containing ethylene glycol (for volatiles), 2N NaOH (for CO_2), and water (carryover) for sampling at 24 h intervals for a total of 48 h. Residual feed was collected for radioanalysis throughout the study. Cages were rinsed with water (times not specified), and the rinse was collected throughout the study into a common container as needed to keep the urine and feces separator functional. At the end of the experiment, cages were rinsed with detergent and water (50/50 v/v), water, and acetone into the same container used for the water rinses. Cages were also wipe-tested to determine the efficiency of the cage rinses.

At study termination (168 h post-dosing), the rats were killed by CO_2 asphyxiation followed by exsanguination. The following tissues were collected for radioanalysis.

Blood (plasma and RBC)[a]	Fat	Liver
Kidney	Muscle	Heart
Lung	Testes	Ovaries
Uterus	Bone/bone marrow[a]	Brain
Spleen	Adrenals	Pituitary
GI tract and contents	Pancreas	Skin
Thyroid	Thymus	Bladder[b]

a Analyzed separately.
b Urine in the bladder at termination was aspirated and combined with the terminal urine sample.

Whole blood was centrifuged to separate plasma and red blood cells. After collection, tissue samples were stored at approximately -10°C until processing and analysis. The residual carcasses were homogenized and stored frozen until analysis.

b. **Pharmacokinetic study:** Four rats/sex/dose level were obtained from the vendor with surgically implanted jugular vein cannulas and were dosed with [^{14}C]-DPX-KJM44 at 25 or 500 mg/kg. Blood samples were collected from the cannulas prior to dosing and at 5, 15, and 30 minutes, and 1, 2, 4, 8, 12, 24, and 30 h post-dosing. Whole blood samples were kept on wet ice immediately following collection; plasma and RBC were separated by centrifugation. RBC were stored at 1-10°C. Plasma samples were held on wet ice or refrigerated if analysis was performed on the same day as collection; if not, plasma was stored frozen at <-10°C. All rats were killed by CO_2 asphyxiation followed by exsanguination and discarded after the final sample collection.

c. **Plasma metabolite profile study:** Three rats/sex were dosed with [^{14}C]-DPX-KJM44 at 500 mg/kg; an additional male and female were used for collection of control plasma. At 30 minutes post-dosing, all rats were killed by CO_2 asphyxiation followed by exsanguination. Whole blood was collected and separated into plasma and RBC. The plasma was stored frozen for metabolite analysis; the RBC were discarded.

d. **Sample analysis:** All samples were analyzed in triplicate, except blood samples from the pharmacokinetic study which were analyzed in duplicate. It was stated that if ongoing analysis of plasma indicated that the [^{14}C]-concentrations were approaching the limit of quantitation, a single maximum aliquot volume per time point was analyzed. The ethylene glycol, NaOH, and water trap contents, plasma, urine, and cage rinses were analyzed directly by LSC. Feces were homogenized in water and combusted; tissues, RBC, food residue, and residual carcasses were homogenized and combusted.

Radioactivity in all samples was quantitated by LSC. It was stated that samples were analyzed for 10 minutes, or until 160,000 disintegrations (0.5%; 2σ) were accumulated, whichever came first. The methods used for quench correction and background determination were not described, the limit of detection was not defined, and oxidizer efficiency was not provided.

e. **Metabolite characterizations:** Urine and fecal samples from the pilot mass balance study and plasma samples from the plasma metabolite profile study were examined for metabolites. An equal volume of methanol was added to each plasma sample (separately); the samples were then mixed, incubated overnight in a freezer to precipitate proteins, mixed again, and centrifuged. The supernatant was removed, an aliquot analyzed by LSC for radioactivity, and the remainder was used for metabolite analysis. Urine samples (pre-dose and 0-24 h) were treated identically as the plasma samples, except the overnight incubation was omitted. Separate fecal homogenates (pre-dose and 0-24 h) were mixed homogeneously and a volume of 50:50 methanol:water (v/v) was added. Five ball bearings were added and the samples were further homogenized. The samples were then centrifuged, and the supernatants were decanted and filtered by syringe through 0.45 µm nylon membranes. An aliquot was analyzed by LSC, and the remainder was used for metabolite analysis.

Quantitation of metabolites and parent was performed using an HPLC coupled to an in-line radioactivity detector. Fractions were collected and appropriate fractions were analyzed for metabolite identification by LC/MS. Identifications were based on retention times and mass fragmentation patterns compared to reference standards. Additionally, control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to assess the stability of the test compound before and after sample preparation at 3 (feces), 10 (urine), and 14 (plasma) days.

3. **Statistics:** Statistical analyses were limited to calculations of mean and standard deviation. Pharmacokinetic parameters were calculated using WinNonlin Professional software (Pharsight Corporation, Mountain View, CA). $C_{max}/2$ (half-maximum concentration) was calculated as one-half C_{max}; $T_{max}/2$ (one-half time to maximum concentration) was estimated by interpolation.

II. RESULTS

A. **CLINICAL OBSERVATIONS:** No clinical signs of toxicity were noted during the cage side observations.

B. **PILOT MASS BALANCE STUDY:** Total recoveries of the administered dose (AD) were 89.2% for the male and 82.6% for the female (Table 2). The test compound was rapidly excreted, with the majority of radioactivity recovered by 24 h post-dosing. The primary route of excretion was the urine (79.27-80.38% AD); fecal radioactivity accounted for 3.06-6.67% AD. Urinary excretion was essentially complete by 48 h post-dosing, while fecal excretion was slightly slower and mostly complete by 72 h post-dosing. No radioactivity was detected in the expired air during the first 48 h; therefore, collection was discontinued. Cage wash accounted for 1.92% and 0.08% AD in the males and females, respectively. A minor amount of radioactivity was detected in the residual feed (0.03-0.07% AD). At 168 h post-dosing, radioactivity was below the limit of detection in the collected tissues except for the male gastrointestinal tract and contents (0.01% AD). Minor amounts of radioactivity were also detected in the residual carcass (0.12-0.14% AD).

TABLE 2. Mean recovery of radioactivity (% administered dose) from rats administered a single 25 mg/kg dose of [^{14}C]-DPX-MAT28.[a]		
Sex	**Male**	**Female**
Urine		
0-24 h	78.80	78.82
24-48 h	1.11	0.33
48-72 h	0.27	0.06
72-168 h	0.20	0.06
Subtotal	80.38	79.27
Feces		
0-24 h	4.57	1.72
24-48 h	1.04	1.03
48-72	0.74	0.26
72-168 h	0.32	0.05
Subtotal	6.67	3.06
Expired air	<LOD	<LOD
Cage wash	1.92	0.08
Residual feed	0.07	0.03
Tissues	0.01[b]	<LOD
Carcass	0.14	0.12
Recovery	**89.19**	**82.56**

a Data were obtained from Tables 2 and 3 on page 38 of the study report.
n=1 rat/sex

b Radioactivity was only detected in the gastrointestinal tract and contents.

<LOD=less than limit of detection

C. **PHARMACOKINETIC STUDIES:** Plasma and RBC pharmacokinetic parameters are presented in Table 3. Absorption of the test compound was rapid, with radioactivity detected in both the plasma and RBC by 5 minutes post-dosing. The plasma elimination half-lives were essentially identical in the males (8.7-13.3 h) and females (10.9-11.6 h), and were unchanged by the 20-fold increase in dose. The mean peak concentrations in plasma of males and females were 20.0 and 16.7 µg equiv./g, respectively, at the 25 mg/kg dose level. These increased by approximately 6-fold to 126.0 and 113.9 µg equiv./g for males and females at the 500 mg/kg dose level. The area under the plasma concentration-time curves ($AUC_{0\rightarrow\infty}$) values were 14.8 and 14.5 µg•h/g for males and females at 25 mg/kg, increasing by 17.2-fold and 16.6-fold to 255.8 and 241.5 µg•h/g for males and females at 500 mg/kg, respectively. The mean time to maximum plasma concentration were 0.3 h for males and females at 25 mg/kg, increasing slightly to 0.4 h and 0.5 h for males and females, respectively, at 500 mg/kg. In RBC, the mean peak concentrations were 7.2 and 8.2 µg equiv./g, respectively, at the 25 mg/kg dose level, increasing by 7.6-fold and 5.9-fold to 54.7 and 48.1 µg equiv./g for males and females at the 500 mg/kg dose level. The ratio of maximum concentration in RBC to plasma ranged from 0.36-0.49, indicating a limited potential for uptake and binding in the RBC.

TABLE 3. Mean (±SD) pharmacokinetic parameters for the plasma and RBC of rats following a single oral dose of [^{14}C]-DPX-MAT28.[a]

Parameter[b]	25 mg/kg		500 mg/kg	
	Male	Female	Male	Female
Plasma				
$t_{½}$ (h)	13.3±0.6	10.9±1.2	8.7±4.1	11.6±4.1
$AUC_{(0\rightarrow\infty)}$ (ug•h/g)	14.8±1.4	14.5±1.5	255.8±59.8	241.5±47.8
$AUC_{(0\rightarrow\infty)}$/dose (ug•h/g/mg)	0.6±0.1	0.6±0.1	0.6±0.1	0.5±0.1
C_{max} (µg equiv./g)	20.0±7.4	16.7±0.8	126.0±14.9	113.9±27.6
$C_{max}/2$ (µg equiv./g)	10.0	8.4	63.0	56.9
t_{Cmax} (h)	0.3±0.0	0.3±0.0	0.4±0.1	0.5±0.0
$t_{Cmax}/2$ (h)	0.6	0.6	1.3	1.5
RBC				
C_{max} (µg equiv./mL)	7.2±2.8	8.2±1.8	54.7±5.1	48.1±10.1
t_{Cmax} (h)	0.3±0.1	0.3±0.0	0.4±0.1	0.6±0.3
Ratio (C_{max} RBC/ C_{max} plasma)	0.36	0.49	0.43	0.42

a Data were obtained from Table 5 on page 40 of the study report. n=4 rats/sex

b Parameters: $t_{½}$ = terminal elimination half-life
AUC = area under concentration-time curve
C_{max} = maximum radioactivity concentration
t_{Cmax} = time to maximum concentration

D. METABOLITE CHARACTERIZATION STUDIES: The plasma samples collected 30 minutes after dosing at 500 mg/kg were initially analyzed for concentration of radioactivity; the mean concentrations were 862 and 868 µM for males and females, respectively. In plasma, urine, and feces, only a single peak identified as DPX-MAT28, the free acid of DPX-KJM44, was observed in each matrix. Identification was confirmed by comparison of mass spectrograph data from the experimental matrices with data obtained from fortified control plasma, and fortified pre-dose urine and feces.

Control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days. No noticeable differences were observed in the urine and feces or in the prepared sample extracts. However, the majority of DPX-KJM44 in plasma converted to DPX-MAT28 when the sample was fortified and analyzed on the same day. Once the plasma was extracted, DPX-KJM44 appeared stable.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: DPX-KJM44 administered by single oral gavage at 25 or 500 mg/kg was rapidly absorbed and excreted as DPX-MAT28. The excretion in urine accounted for the majority of the dose within the first 24 hours after dosing. No radioactivity was detected in exhaled breath collected for 0-48 hours or in tissues collected at 168 hours, except for a minor percentage in the male rat gastrointestinal tract and carcass and female rat carcass. Definitive pharmacokinetic data in plasma indicated rapid uptake and terminal elimination. Although the peak concentration increased about 6- to 7-fold from low to high dose, the AUC values increased 17-fold, nearly proportional with dose. The

metabolite profile in plasma at 0.5 hours and in pilot urine and feces samples 0-24 hours after dosing were confirmed to contain only DPX-MAT28, the free acid of DPX-KJM44.

B. REVIEWER COMMENTS: No clinical signs of toxicity were noted during the cage side observations.

Absorption of the test compound was rapid, with radioactivity detected in both the plasma and RBC by 5 minutes post-dosing. The plasma elimination half-lives were similar in the males and females, and were unchanged by the 20-fold increase in dose. The mean peak concentrations in plasma of males and females increased by approximately 6-fold from 25 mg/kg to 500 mg/kg. The area under the plasma concentration-time curves ($AUC_{0\rightarrow\infty}$) increasing by 17-fold for males and females from 25 mg/kg to 500 mg/kg. The mean time to maximum plasma concentration increased very slightly from 25 mg/kg to 500 mg/kg. In RBC, the mean peak concentrations increased by 8-fold and 6-fold for males and females, respectively, from 25 mg/kg to 500 mg/kg. The ratio of maximum concentration in RBC to plasma indicated a limited potential for uptake and binding in the RBC.

Total recoveries were 89.2% AD for the male and 82.6% AD for the female. The test compound was rapidly excreted, with the majority of radioactivity recovered by 24 h post-dosing. The primary route of excretion was the urine; urinary excretion was essentially complete by 48 h post-dosing. Fecal excretion was slightly slower and mostly complete by 72 h post-dosing. No radioactivity was detected in the expired air during the first 48 h. Cage wash accounted for 1.92% and 0.08% AD in the males and females, respectively. A minor amount of radioactivity was detected in the residual feed. At 168 h post-dosing, radioactivity was below the limit of detection in the collected tissues except for the male gastrointestinal tract and contents (0.01% AD). Minor amounts of radioactivity were also detected in the residual carcass (0.12-0.14% AD).

The plasma samples collected 30 minutes after dosing at 500 mg/kg were initially analyzed for concentration of radioactivity; the mean concentrations were 862 and 868 μM/mL or what unit for males and females, respectively. In plasma, urine, and feces, only a single peak identified as DPX-MAT28, the free acid of DPX-KJM44, was observed in each matrix. Identification was confirmed by comparison of mass spectrograph data from the experimental matrices with data obtained from fortified control plasma, and fortified pre-dose urine and feces. In a concurrently reviewed subchronic oral toxicity study in rats (MRID 47573403), a metabolite (IN-LTX69) was quantified in plasma; however, this metabolite was not detected in the present study following a single dose of the test compound. Additionally, control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days. No noticeable differences were observed in the urine and feces or in the prepared sample extracts. However, the majority of DPX-KJM44 in plasma converted to DPX-MAT28 when the sample was fortified and analyzed on the same day. Once the plasma was extracted, DPX-KJM44 appeared stable.

This metabolism study is classified **acceptable/guideline** and satisfies the guideline requirement for a Tier 1 metabolism study [OPPTS 870.7485, OECD 417] in rats.

C. **STUDY DEFICIENCIES:** The following deficiencies were observed and do not affect the acceptability of the study:

- Concentration, radiochemical purity, homogeneity and stability data for the dosing solutions were not provided. However, the actual mean doses presented were sufficiently close to the nominal doses to assure the reviewers that the concentration and homogeneity of the dosing solutions were within acceptable limits. Additionally, control pooled plasma samples and pre-dose urine and fecal samples were fortified with radiolabeled dosing solution to determine stability at 3 (feces), 10 (urine), and 14 (plasma) days; no noticeable differences were observed in the urine and feces or in the prepared sample extracts. However, the majority of DPX-KJM44 in plasma converted to DPX-MAT28 when the sample was fortified and analyzed on the same day. Once the plasma was extracted, DPX-KJM44 appeared stable.

- The methods used for quench correction and background determination were not described, the limit of detection was not defined, and oxidizer efficiency was not provided.

- The pilot mass balance study was conducted on only one animal/sex.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.7800; Immunotoxicity Study in Rats

Work Assignment No. 5-01-209 P (MRID 47560025)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
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Prepared by
Pesticides Health Effects Group
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Primary Reviewer: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 03/30/09
Secondary Reviewer: John W. Allran, M.S.	Signature: John W Allran Date: 03/30/09
Program Manager: Michael E. Viana, Ph.D., D.A.B.T.	Signature: Michael E Viana Date: 03/30/09
Quality Assurance: Steven Brecher, Ph.D., D.A.B.T.	Signature: Steven Brecher Date: 03/30/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/2008
EPA Reviewer: Marquea D. King, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/08
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** Stephen C. Kapsan Jr
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2008

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity [feeding] - rats; OPPTS 870.7800

PC CODE: 288008 **DP BARCODE:** D361256
TXR #: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Hoban, D. (2008) DPX-MAT28 technical: 28-day immunotoxicity feeding study in male rats. E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE. Laboratory Project ID.: DuPont-22794, June 18, 2008. MRID 47560025. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 47560025) DPX-MAT28 (Aminocyclopyrachlor; 92.2% a.i.; Batch No. DPX-MAT28-009) was administered to ten male Sprague Dawley (Crl:CD[SD]) rats/dose group in the diet at dose levels of 0, 600, 6,000 or 18,000 ppm (equivalent to 0, 42, 407, and 1277 mg/kg bw/day) for 28 days. An additional group of five rats were dosed by intraperitoneal injection (10 mL/kg) with 25 mg/kg cyclophosphamide monohydrate to serve as positive controls. Systemic toxicity parameters were evaluated, and immunotoxicity was investigated by performing an enzyme-linked immunoabsorbant assay (ELISA) to measure serum IgM responses to sheep red blood cells (sRBC) following immunization with sRBC.

Systemic toxicity was not observed. There were no effects of treatment on mortality, clinical signs, body weights, body weight gains, food consumption, food efficiency, organ weights, or gross pathology at any dose level.

The LOAEL for systemic toxicity was not observed. The NOAEL for systemic toxicity is 18,000 ppm (equivalent to 1277 mg/kg bw/day in males).

There was no evidence of immunotoxicity. No effects of treatment were observed on the humoral immune response as measured by the primary serum IgM antibody response to the T-

dependent antigen, sheep erythrocytes. Spleen and thymus weights were unaffected by treatment.

Positive control rats demonstrated an 82% decrease in Log_2 titer compared to concurrent controls, demonstrating the ability of this ELISA to detect suppression of the humoral immune response.

The LOAEL for immunotoxicity was not observed. The NOAEL for immunotoxicity is 18,000 ppm (equivalent to 1277 mg/kg bw/day in males).

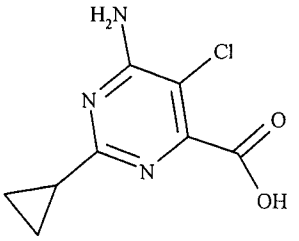
This immunotoxicity study is classified **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.7800) for an immunotoxicity study in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** DPX-MAT28 technical (Aminocyclopyrachlor)

Description:	White solid
Batch #:	DPX-MAT28-009
Purity:	92.2% a.i.
Compound Stability:	Stable in the diet for up to 14 days when stored at room temperature or 21 days when stored refrigerated
CAS # of TGAI:	858956-08-8
Structure:	

2. **Vehicle and/or positive control:** Test compound was administered in the diet; cyclophosphamide monohydrate was administered by intraperitoneal injection as a positive control.

3. **Test animals:**

Species:	Rats (males only)	
Strain:	Sprague Dawley (Crl:CD[SD])	
Age/weight at study initiation:	8 weeks; 233.0-273.1 g	
Source:	Charles River Laboratories (Raleigh, NC)	
Housing:	Individually in stainless steel, wire mesh cages suspended over cage board	
Diet:	Certified Rodent LabDiet® 5002 (PMI® Nutrition International, LLC, St. Louis, MO), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	Not provided
	Photoperiod:	12 h light/12 h dark
Acclimation period:	Six days	

B. STUDY DESIGN

1. **In life dates:** Start: 07/12/07 End: Approximately 08/09/07

2. **Animal assignment**: Animals were randomly assigned, stratified by body weight, to the test groups noted in Table 1. The weight variation of the selected animals did not exceed ±20% of the mean weight.

TABLE 1: Study design[a]			
Test group	**Dietary concentration (ppm)**	**Intake (mg/kg/day)**	**# Males**
Control	0	0	10
Low	600	42	10
Mid	6000	407	10
High	18,000	1277	10
Cyclophosphamide[b]	NA	25 mg/kg	5

a Data were obtained from page 11 and Table 5 on page 28 of the study report. Dietary formulations were adjusted for test compound purity.

b Cyclophosphamide monohydrate (25 mg/kg) was injected intraperitoneally on Days 22-27 at a dose volume of 10 mL/kg as a positive control.

3. **Dose selection:** It was stated that the doses were selected based on the results of a previously performed dietary subchronic toxicity study in rats (concurrently reviewed; MRID 47573403). In this study, fifteen Sprague Dawley (Crl:CD[SD]) rats/sex/group) were administered diets that contained 0, 600, 2000, 6000, or 18,000 ppm DPX-MAT28 (equivalent to 0/0, 35/45, 114/146, 349/448, and 1045/1425 mg/kg/day in males/females), for approximately 90 days.

   No test substance-related deaths occurred, and there were no effects of treatment on clinical signs, food consumption, ophthalmological or neurobehavioral observations, clinical pathology parameters, organ weights, or gross, microscopic, or neuropathology.

   Test substance-related effects on body weight parameters, body weight gain, and food efficiency were observed in male and female rats fed 18,000 ppm. Final (Day 91) mean body weights in males and females were 9% and 8%, respectively, lower than control. Overall (Days 0-91) mean body weight gains and food efficiency in males and females were 15% and 18% and 11% and 17%, respectively, lower compared to control.

   The LOAEL was 18,000 ppm (equivalent to 1045/1425 mg/kg/day in males/females), based on effects on body weight parameters and food efficiency in both sexes. The NOAEL was 6000 ppm (equivalent to 349/448 mg/kg/day in males/females).

4. **Test diet preparation and analysis:** Dietary formulations were prepared by mixing the appropriate amount of the test substance (adjusted for purity) for each concentration with basal diet. Diets were prepared every other week and were stored refrigerated until use. Diets stored beyond the stability period (21 days refrigerated) were not presented to the animals. Homogeneity (top, middle, bottom) and concentration were determined in all dose levels of the initial dietary preparation. Stability analyses were performed in a concurrently reviewed subchronic toxicity study (MRID 47573403) on formulations containing 300 and 18,000 ppm. Stability was determined following room temperature storage for up to 14 days and refrigerated storage for up to 21 days. Concentration analyses were also performed on all dose levels of a second preparation.

Results

Homogeneity analysis (%CV): 1.38-2.36%

Stability analysis (% of Day 0): 95.9-107% after room temperature storage for 14 days
99.4-117% after refrigerated storage for 21 days

Concentration analysis (% of nominal): 85.2-99.9%

The analytical data indicate that the mixing procedure was adequate, and that the variation between the target and actual dosage to the study animals was marginally acceptable.

5. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure		
	Preliminary tests	Preliminary test is not significant	Preliminary test is significant
Body weight, body weight gain, food consumption, food efficiency, and organ weights	Levine's test for homogeneity and Shapiro-Wilk test for normality[b]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Humoral immune function data[a]	Bartlett's Chi Square test	One-way analysis of variance followed by Dunnett's test	Gehan-Wilcoxon test

a Sheep red blood cell- (SRBC) specific serum IgM antibody titer data were transformed to Log_2 to obtain normality or homogeneous variances.

b If the Shapiro-Wilk test was not significant but Levine's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was used, followed by Dunn's test if necessary.

Humoral immune function data were first tested for homogeneity of variances using the Bartlett's Chi Square test. Homogeneous data were evaluated by a parametric one-way analysis of variance. When significant differences occurred, treatment groups were compared to the vehicle control group using Dunnett's t test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, treatment groups were compared to the vehicle control group using the Gehan-Wilcoxon test when appropriate. Jonckheere's test was used to test for treatment level-related trends across the vehicle control and treatment groups. The positive control was compared to the vehicle control group using Student's t test. Statistical significance was denoted at $p \leq 0.05$ (and $p \leq 0.01$ for Student's t test). The statistical methods were considered appropriate.

C. METHODS

1. **Observations:** Cage-side inspections were performed at least once daily for mortality, morbidity, and abnormal behavior and/or appearance throughout the study. Additionally, at each weighing, individual rats were handled and examined for abnormal behavior and appearance.

2. **Body weight:** Animals were weighed prior to the initiation of treatment, on Days 0, 7, 14, 21, and 28, and on the day of sacrifice. Mean body weight gains were calculated for each weighing period and for the overall (Days 0-28) treatment period.

3. **Food consumption, food efficiency, and compound intake:** Food consumption (g/rat/day) was determined by weighing each feeder at the beginning and end of each body weight interval and subtracting the final weight and spillage from the initial weight. Food consumption, food efficiency (g body weight gained ÷ g food consumed), and compound intake were presented for each body weight interval and for the overall (Day 0-28) treatment period.

4. **Sacrifice and pathology:** Rats were euthanized on Day 28 by carbon dioxide anesthesia followed by exsanguination. The order of termination was stratified across groups.

a. **Gross pathology:** All animals were subjected to a thorough gross pathological examination.

b. **Organ weights:** Absolute, relative to body, and relative to brain spleen, thymus, and brain weights were recorded from all test substance-treated animals. Organs from the positive control animals were not weighed.

5. **Immunotoxicity:** Immunotoxicity was assessed with an enzyme-linked immunoabsorbant assay (ELISA) to measure serum IgM responses to sheep red blood cells (sRBC). On Day 22, all animals were immunized by intravenous injection in the lateral tail vein with 0.5 mL of sRBC at a concentration of 4 x 10^8 sRBC/mL. Following immunization with sRBC, rats in the positive control group were injected intraperitoneally with the known immunosuppressive agent, cyclophosphamide monohydrate (in deionized water), at 25 mg/kg (10 mL/kg) for six consecutive days. At euthanasia, approximately 4 mL of blood were collected from the abdominal *vena cava* of all rats and processed to serum for ELISA analysis.

The ELISA was performed as follows. The day prior to the assay, 96-well plates were prepared by coating the wells with an sRBC membrane preparation in phosphate-buffered saline. The plates were incubated refrigerated overnight, then rinsed, blocked for 1 h at room temperature, and rinsed. Appropriately diluted test serum and ten serial 1:2 dilutions were applied and incubated 1 h. The plates were rinsed and goat anti-rat IgM horseradish peroxidase-conjugated antibody was added. The plates were rinsed and the enzyme substrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), was added. The optical density was read at 405 nm after 45 minutes of incubation using a microtiter plate reader.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All rats survived to scheduled termination.

2. **Clinical signs of toxicity:** No clinical signs of toxicity were observed. The most common finding in the treated groups was hair loss, which was not dependent on dose.

B. **BODY WEIGHT AND WEIGHT GAIN:** Body weight and overall body weight gain data are presented in Table 2. There were no effects of treatment observed on body weights or body weight gains in any dose group.

Positive control rats administered 25 mg/kg cyclophosphamide monohydrate for six consecutive days beginning on Day 23 demonstrated a 9% decrease ($p \leq 0.05$) in body weight compared to controls on Day 28, associated with a body weight loss (-16.7 g treated vs. +26.4g concurrent controls; $p \leq 0.05$) for Days 21-28. Overall body weight gains (Days 0-28) were also decreased ($p \leq 0.05$) by 28% in this group.

TABLE 2: Selected mean (±SD) body weights and body weight gains (g) in male rats administered DPX-MAT28 in the diet for up to 28 days.[a]

Parameter/time point		Dose (ppm) 0	600	6000	18,000	Positive controls[b]
		Males				
Body weight	Day 0	250.4±9.1	251.7±9.4	252.0±5.2	251.4±7.5	259.4±9.3
	Day 7	314.2±8.9	312.7±16.6	310.0±13.1	307.9±10.8	318.9±11.1
	Day 14	357.8±14.8	355.6±23.2	349.0±17.9	351.3±16.7	361.9±13.5
	Day 21	399.7±19.8	398.5±29.3	390.1±23.5	387.2±23.8	403.5±16.2
	Day 28	426.2±22.6	428.1±34.6	416.4±26.0	411.8±27.9	386.8±20.5* (↓9)
Body weight gain	Day 0-28	175.8±19.3	176.4±27.2	164.3±23.0	160.4±24.8	127.4±13.5* (↓28)

a Data were obtained from Tables 1 and 2 on pages 24-25 of the study report. n=10
b Positive controls were treated with 25 mg/kg cyclophosphamide i.p. for 6 consecutive days beginning on Day 23; n=5
* Statistically different from controls; $p \leq 0.05$

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE

1. **Food consumption and food efficiency:** There were no adverse, treatment-related effects on food consumption or food efficiency. Food efficiency was decreased ($p \leq 0.05$) by 17% on Days 14-21 in the 18,000 ppm group, but this transient decrease was not considered adverse. Positive control rats demonstrated an 18% decrease ($p \leq 0.05$) in food consumption during Days 21-28, negative ($p \leq 0.05$) food efficiency during Days 21-28 (corresponding to the body weight loss for this interval), and a 27% decrease ($p \leq 0.05$) in overall food efficiency.

2. **Compound consumption:** Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

D. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No treatment-related effects were observed on the mean absolute or relative (to body) brain, spleen, or thymus weights or relative (to brain) spleen or thymus weights in any dose group. Relative (to body) spleen weights were increased ($p \leq 0.05$) by 14% in the 6000 ppm group, but this finding was unrelated to dose.

2. **Gross pathology:** No gross lesions were observed in any of the animals at necropsy.

E. **IMMUNOTOXICITY:** Immunotoxicity results are presented in Table 3. No effects of treatment were observed on the humoral immune response as measured by the primary serum IgM antibody response to the T-dependent antigen, sheep erythrocytes.

Positive control rats demonstrated an 82% decrease ($p \leq 0.01$) in Log_2 titer compared to concurrent controls, demonstrating the ability of this ELISA to detect suppression of the humoral immune response.

TABLE 3. Mean (±SE) serum primary IgM antibody titers (Log_2) to sheep red blood cells in male rats administered DPX-MAT28 in the diet for up to 28 days.[a]

Titer (Log_2)	Dose (ppm)				
	0	600	6000	18,000	Positive controls[b]
Titer	8.565±0.329	8.951±0.267	9.618±0.263	9.060±0.208	1.519±0.930** (↓82)

a Data were obtained from Appendix F, Table 1, on page 83 of the study report. n=10
b Positive controls were treated with 25 mg/kg cyclophosphamide for 6 consecutive days beginning on Day 22; n=5
** Statistically different from controls; $p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** Under the conditions of this study, the NOAEL for DPX-MAT28 was 18,000 ppm (equivalent to 1277 mg/kg/day). The NOAEL is based on a lack of adverse test substance-related effects on any in-life or anatomic pathology parameter or on the humoral immune response in male rats fed up to 18,000 ppm.

B. **REVIEWER COMMENTS:** Systemic toxicity was not observed. There were no effects of treatment on mortality, clinical signs, body weights, body weight gains, food consumption, food efficiency, organ weights, or gross pathology at any dose level.

The LOAEL for systemic toxicity was not observed. The NOAEL for systemic toxicity is 18,000 ppm (equivalent to 1277 mg/kg bw/day in males).

There was no evidence of immunotoxicity. The immune system is not a target organ based on the lack of effects of treatment on the humoral immune response as measured by the primary serum IgM antibody response to the T-dependent antigen, sheep erythrocytes. Spleen and thymus weights were also unaffected by treatment.

Positive control rats demonstrated an 82% decrease ($p \leq 0.01$) in Log_2 titer compared to concurrent controls, demonstrating the ability of this ELISA to detect suppression of the humoral immune response.

The LOAEL for immunotoxicity was not observed. The NOAEL for immunotoxicity is 18,000 ppm (equivalent to 1277 mg/kg bw/day in males).

C. **STUDY DEFICIENCIES:**
No deficiencies were noted.

DATA EVALUATION RECORD

AMINOCYCLOPYRACHLOR (DPX-MAT28)

Study Type: OPPTS 870.7800; Immunotoxicity Study in Mice

Work Assignment No. 5-01-209 Q (MRID 47560026)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
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Date: 03/30/09

Quality Assurance:
Steven Brecher, Ph.D., D.A.B.T.
Signature: Steven Brecher
Date: 03/30/09

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Jessica P. Ryman, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/2009
EPA Reviewer: Marquea D. King, Ph.D. **Signature:**
Risk Assessment Branch 4, Health Effects Division (7509P) **Date:** 10/14/09
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:**
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 10/15/2009

DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity [feeding] - mice; OPPTS 870.7800

PC CODE: 288008 **DP BARCODE:** D361080
TXR #: 0055188

TEST MATERIAL (PURITY): Aminocyclopyrachlor (92.2% a.i.)

SYNONYMS: DPX-MAT28; 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid

CITATION: Hoban, D. (2008) DPX-MAT28 technical: 28-day immunotoxicity feeding study in male mice. E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE. Laboratory Project ID.: DuPont-22795, June 18, 2008. MRID 47560026. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company, Wilmington, DE

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 47560026) DPX-MAT28 (Aminocyclopyrachlor; 92.2% a.i.; Batch Nos. DPX-MAT28-009) was administered to ten male CD-1 (Crl:CD[ICR]) mice/dose group in the diet at dose levels of 0, 300, 3,000 or 7,000 ppm (equivalent to 0, 45, 425, and 1056 mg/kg bw/day) for 28 days. An additional group of five mice were dosed by intraperitoneal injection (10 mL/kg) with 25 mg/kg cyclophosphamide monohydrate to serve as positive controls. Systemic toxicity parameters were evaluated, and immunotoxicity was investigated by performing an enzyme-linked immunoabsorbant assay (ELISA) to measure serum IgM responses to sheep red blood cells (sRBC) following immunization with sRBC.

Systemic toxicity was not observed. There were no effects of treatment on mortality, clinical signs, body weights, body weight gains, food consumption, food efficiency, organ weights, or gross pathology at any dose level.

The LOAEL for systemic toxicity was not observed. The NOAEL for systemic toxicity is 7000 ppm (equivalent to 1056 mg/kg bw/day in males).

There was no evidence of immunotoxicity. No effects of treatment were observed on the humoral immune response as measured by the primary serum IgM antibody response to the T-

dependent antigen, sheep erythrocytes. Spleen and thymus weights were unaffected by treatment.

Positive control mice demonstrated a 73% decrease in Log_2 titer compared to concurrent controls, demonstrating the ability of this ELISA to detect suppression of the humoral immune response.

The LOAEL for immunotoxicity was not observed. The NOAEL for immunotoxicity is 7000 ppm (equivalent to 1056 mg/kg bw/day in males).

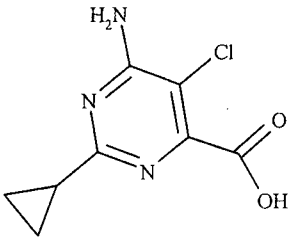
This immunotoxicity study is classified **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.7800) for an immunotoxicity study in mice.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28 technical (Aminocyclopyrachlor)

Description:	White solid
Batch #:	DPX-MAT28-009
Purity:	92.2% a.i.
Compound Stability:	Stable in the diet for up to 14 days when stored at room temperature or 21 days when stored refrigerated
CAS # of TGAI:	858956-08-8
Structure:	

2. Vehicle and/or positive control: Test compound was administered in the diet; cyclophosphamide monohydrate was administered by intraperitoneal injection as a positive control.

3. Test animals:

Species:	Mice (males only)
Strain:	CD-1 (Crl:CD[ICR])
Age/weight at study initiation:	8 weeks; 29.4-34.5 g
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Individually in stainless steel, wire mesh cages suspended over cage board
Diet:	Certified Rodent LabDiet® 5002 (PMI® Nutrition International, LLC, St. Louis, MO), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70% **Air changes:** Not provided **Photoperiod:** 12 h light/12 h dark
Acclimation period:	Eight days

B. STUDY DESIGN

1. In life dates: Start: 08/21/07 End: Approximately 09/18/07

2. Animal assignment: Animals were randomly assigned, stratified by body weight, to the test groups noted in Table 1. The weight variation of the selected animals did not exceed ±20% of the mean weight.

TABLE 1: Study design[a]			
Test group	**Dietary concentration (ppm)**	**Intake (mg/kg/day)**	**# Males**
Control	0	0	10
Low	300	45	10
Mid	3000	425	10
High	7000	1056	10
Cyclophosphamide[b]	NA	25 mg/kg	5

a Data were obtained from page 11 and Table 5 on page 28 of the study report. Dietary formulations were adjusted for test compound purity.

b Cyclophosphamide monohydrate (25 mg/kg) was injected intraperitoneally on Days 24-28 at a dose volume of 10 mL/kg as a positive control.

3. **Dose selection:** It was stated that the doses were selected based on the results of a previously performed dietary subchronic toxicity study in mice (concurrently reviewed; MRIDs 47560010 and 47560011). In this study, DPX-MAT28 was administered to 15 CD-1 mice/sex/dose group in the diet at dose levels of 0, 300, 1000, 3000, or 7000 ppm (equivalent to 0/0, 47/61, 154/230, 459/649, and 1088/1623, respectively) for approximately 90 days (95/96 days in males/females). At Day 60, blood was collected from the orbital sinus or tail vein of each mouse from the satellite groups (5/sex/concenctration) for the determination of plasma concentration of the test substance (DPX-MAT28) and/or the metabolite (IN-LXT69).

   There were no effects of treatment on mortality, clinical signs, body weights, body weight gains, food consumption, food efficiency, ophthalmoscopic examinations, hematology, clinical chemistry, organ weights, gross pathology, or histopathology.

   In the satellite animals, plasma concentrations of DPX-MAT28 were dose-dependently increased in all treated groups, with concentrations of 157, 480, 1376, and 2484 ng/mL in the males and 121, 436, 1249, and 2313 ng/mL in the females fed the 300, 1000, 3000, and 7000 ppm diets respectively. Slightly higher plasma concentrations of the parent were noted in the males than in the females at all dose levels. None of the plasma samples detected the metabolite IN-LXT69 concentrations above the limit of quantitation.

   The LOAEL was not observed. The NOAEL is 7000 ppm (equivalent to 1088/1623 mg/kg/day in males/females).

4. **Test diet preparation and analysis:** Dietary formulations were prepared by mixing the appropriate amount of the test substance (adjusted for purity) for each concentration with basal diet. Diets were prepared every other week and were stored refrigerated until use. Diets stored beyond the stability period (21 days refrigerated) were not presented to the animals. Homogeneity (top, middle, bottom) and concentration were determined in all dose levels of the initial dietary preparation. Stability analyses were performed in a concurrently reviewed subchronic toxicity study (MRID 47573403) on formulations containing 300 and 18,000 ppm. Stability was determined following room temperature storage for up to 14 days and refrigerated storage for up to 21 days. Concentration analyses were also performed on all dose levels of a second preparation.

Results

Homogeneity analysis (%CV): 2-9%

Stability analysis (% of Day 0): 95.9-107% after room temperature storage for 14 days
99.4-117% after refrigerated storage for 21 days

Concentration analysis (% of nominal): 84.2-116%

The analytical data indicate that the mixing procedure was marginally adequate, and that the variation between the target and actual dosage to the study animals was marginally acceptable.

5. **Statistics:** The following statistical procedures were used:

Parameter	Statistical procedure		
	Preliminary tests	Preliminary test is not significant	Preliminary test is significant
Body weight, body weight gain, food consumption, food efficiency, and organ weights	Levine's test for homogeneity and Shapiro-Wilk test for normality[b]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's test
Humoral immune function data[a]	Bartlett's Chi Square test	One-way analysis of variance followed by Dunnett's test	Gehan-Wilcoxon test

a Sheep red blood cell- (SRBC) specific serum IgM antibody titer data were transformed to Log_2 to obtain normality or homogeneous variances.

b If the Shapiro-Wilk test was not significant but Levine's test was significant, a robust version of Dunnett's test was used. If the Shapiro-Wilk test was significant, Kruskal-Wallis test was used, followed by Dunn's test if necessary.

Humoral immune function data were first tested for homogeneity of variances using the Bartlett's Chi Square test. Homogeneous data were evaluated by a parametric one-way analysis of variance. When significant differences occurred, treatment groups were compared to the vehicle control group using Dunnett's t test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, treatment groups were compared to the vehicle control group using the Gehan-Wilcoxon test when appropriate. Jonckheere's test was used to test for treatment level-related trends across the vehicle control and treatment groups. The positive control was compared to the vehicle control group using Student's t test. Statistical significance was denoted at $p \leq 0.05$ (and $p \leq 0.01$ for Student's t test). The statistical methods were considered appropriate.

C. METHODS

1. **Observations:** Cage-side inspections were performed at least once daily for mortality, morbidity, and abnormal behavior and/or appearance throughout the study. Additionally, at each weighing, individual were handled and examined for abnormal behavior and appearance.

2. **Body weight:** Animals were weighed prior to the initiation of treatment, on Days 0, 7, 14, 21, and 28, and on the day of sacrifice. Mean body weight gains were calculated for each weighing period and for the overall (Days 0-28) treatment period.

3. **Food consumption, food efficiency, and compound intake:** Food consumption (g/mouse/day) was determined by weighing each feeder at the beginning and end of each body weight interval and subtracting the final weight and spillage from the initial weight. Food consumption, food efficiency (g body weight gained ÷ g food consumed), and compound intake were presented for each body weight interval and for the overall (Day 0-28) treatment period.

4. **Sacrifice and pathology:** Mice were euthanized on Day 28 by carbon dioxide anesthesia followed by exsanguination. The order of termination was stratified across groups.

a. **Gross pathology:** All animals were subjected to a thorough gross pathological examination.

b. **Organ weights:** Absolute, relative to body, and relative to brain spleen, thymus, and brain weights were recorded from all test substance-treated animals. Organs from the positive control animals were not weighed.

5. **Immunotoxicity:** Immunotoxicity was assessed with an enzyme-linked immunoabsorbant assay (ELISA) to measure serum IgM responses to sheep red blood cells (sRBC). On Day 23, all animals were immunized by intravenous injection in the lateral tail vein with 0.2 mL of sRBC at a concentration of 5 x 10^8 sRBC/mL. Following immunization with sRBC, mice in the positive control group were injected intraperitoneally with the known immunosuppressive agent, cyclophosphamide monohydrate (in deionized water), at 25 mg/kg (10 mL/kg) for five consecutive days. At euthanasia, approximately 1 mL of blood was collected from the abdominal *vena cava* of each mouse and processed to serum for ELISA analysis.

The ELISA was performed as follows. The day prior to the assay, 96-well plates were prepared by coating the wells with an sRBC membrane preparation in phosphate-buffered saline. The plates were incubated refrigerated overnight, then rinsed, blocked for 1 h at room temperature, and rinsed. Appropriately diluted test serum and ten serial 1:2 dilutions were applied and incubated 1 h. The plates were rinsed, and goat anti-mouse IgM horseradish peroxidase-conjugated antibody was added. The plates were rinsed, and the enzyme substrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), was added. The optical density was read at 405 nm after 45 minutes of incubation using a microtiter plate reader.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All mice survived to scheduled termination.

2. **Clinical signs of toxicity:** No clinical signs of toxicity were observed.

B. **BODY WEIGHT AND WEIGHT GAIN:** Body weight and overall body weight gain data are presented in Table 2. There were no effects of treatment observed on body weights or body weight gains in any dose group or in the positive controls.

TABLE 2: Selected mean (±SD) body weights and body weight gains (g) in male mice administered DPX-MAT28 in the diet for up to 28 days.[a]

Parameter/time point		Dose (ppm) 0	300	3000	7000	Positive controls[b]
		Males				
Body weight	Day 0	31.7±0.9	31.9±1.8	32.3±1.6	32.3±1.1	31.1±1.1
	Day 7	32.3±0.9	32.7±2.1	33.1±2.1	32.8±1.3	32.5±1.0
	Day 14	32.7±1.1	33.2±2.4	33.9±2.3	33.6±1.6	33.2±1.3
	Day 21	34.2±1.3	34.7±2.7	35.3±2.6	34.7±1.8	34.7±1.5
	Day 28	35.4±1.5	35.6±2.9	36.4±2.3	35.6±1.9	35.4±1.5
Body weight gain	Day 0-28	3.7±1.0	3.7±1.7	4.1±1.1	3.4±1.4	4.3±1.8

a Data were obtained from Tables 1 and 2 on pages 24-25 of the study report. n=10
b Positive controls were treated with 25 mg/kg cyclophosphamide i.p. for 5 consecutive days beginning on Day 23; n=5
* Statistically different from controls; $p \leq 0.05$

C. FOOD CONSUMPTION, FOOD EFFICIENCY, AND COMPOUND INTAKE

1. **Food consumption and food efficiency:** There were no adverse, treatment-related effects on food consumption or food efficiency. Food consumption was increased ($p \leq 0.05$) by 9% on Days 21-28 in the 7000 ppm group, but this minor, transient increase was not considered adverse.

2. **Compound consumption:** Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

D. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No treatment-related effects were observed on the mean absolute or relative (to body) brain, spleen, or thymus weights or relative (to brain) spleen or thymus weights in any dose group.

2. **Gross pathology:** No gross lesions were observed in any of the animals at necropsy.

E. **IMMUNOTOXICITY:** Immunotoxicity results are presented in Table 3. No effects of treatment were observed on the humoral immune response as measured by the primary serum IgM antibody response to the T-dependent antigen, sheep erythrocytes.

Positive control mice demonstrated a 73% decrease ($p \leq 0.01$) in Log_2 titer compared to concurrent controls, demonstrating the ability of this ELISA to detect suppression of the humoral immune response.

TABLE 3. Mean (±SE) serum primary IgM antibody titers (Log_2) to sheep red blood cells in male mice administered DPX-MAT28 in the diet for up to 28 days.[a]

Titer (Log_2)	Dose (ppm)				
	0	300	3000	7000	Positive controls[b]
Titer	7.781±0.207	7.812±0.201	8.027±0.309	7.849±0.256	2.093±0.990** (↓73)

a Data were obtained from Appendix F, Table 1, on page 89 of the study report. n=10
b Positive controls were treated with 25 mg/kg cyclophosphamide for 5 consecutive days beginning on Day 24; n=5
** Statistically different from controls; $p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS CONCLUSIONS:** Under the conditions of this study, the NOAEL for DPX-MAT28 was 7000 ppm (equivalent to 1056 mg/kg/day). The NOAEL is based on a lack of adverse test substance-related effects on any in-life or anatomic pathology parameter or on the humoral immune response in male mice fed up to 7000 ppm.

B. **REVIEWER COMMENTS:** Systemic toxicity was not observed. There were no effects of treatment on mortality, clinical signs, body weights, body weight gains, food consumption, food efficiency, organ weights, or gross pathology at any dose level.

The LOAEL for systemic toxicity was not observed. The NOAEL for systemic toxicity is 7000 ppm (equivalent to 1056 mg/kg bw/day in males).

There was no evidence of immunotoxicity. No effects of treatment were observed on the humoral immune response as measured by the primary serum IgM antibody response to the T-dependent antigen, sheep erythrocytes. Spleen and thymus weights were unaffected by treatment.

Positive control mice demonstrated an 73% decrease ($p \leq 0.01$) in Log_2 titer compared to concurrent controls, demonstrating the ability of this ELISA to detect suppression of the humoral immune response.

The LOAEL for immunotoxicity was not observed. The NOAEL for immunotoxicity is 7000 ppm (equivalent to 1056 mg/kg bw/day in males).

C. **STUDY DEFICIENCIES:** No deficiencies were noted.

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R178808

Chemical Name: 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
4-Pyrimidinecarboxylic acid, 6-amino-5-chloro-2-cyclopropyl-, methyl ester

PC Code: 288008

288009

HED File Code: 13000 Tox Reviews
Memo Date: 10/15/2009
File ID: 00000000
Accession #: 000-00-0132

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